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The Barge *Ocean 250* Gasoline Spill

Carolyn A. Griswold, Editor

November 1981

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National Marine Fisheries Service
Environmental Protection Agency
University of Rhode Island
Energy Resources Company, Inc.

U.S. DEPARTMENT OF COMMERCE
Malcolm Baldrige, Secretary

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The Barge *Ocean 250* Gasoline Spill

CAROLYN A. GRISWOLD,[1] Editor

ABSTRACT

On 16 March 1978, the barge *Ocean 250* grounded on Watch Hill Reef 1,006 m off Watch Hill, Rhode Island. An estimated 2.6 million liters of gasoline was spilled into Block Island Sound.

Results of cytogenetic analyses indicated maximum damage occurred in fish eggs collected in plankton and neuston samples in the spill area. Membrane or embryo damage occurred in up to 100% of the fourbeard rockling, *Enchelyopus cimbrius,* and yellowtail flounder, *Limanda ferruginea,* eggs collected over a 4 day period following the spill. Low levels (12 ppb) of hydrocarbons analyzed in the gasoline range were found in the water column at stations in the spill area 36-40 hours after the spill first began. Zooplankton samples collected from the same area showed traces of hydrocarbons from the gasoline range as did two species of benthic invertebrates, the sea scallop, *Placopecten magellanicus,* and the hard shell clam, *Mercenaria mercenaria.* Twenty-three fish samples representing 10 species were anlayzed. Five showed levels twice that of the control sample taken from Fox Island, Narragansett Bay. There was no apparent damage to benthic communities, and analyses of zooplankton communities at the time of the spill and 3 weeks later showed normal patterns of species composition and abundance.

With the exception of localized damage to fish eggs, there was no apparent discernible damage to fish or invertebrate populations in the area immediately following the spill, and although there were measurable amounts of gasoline hydrocarbon components in a small number of water, fish, and invertebrate samples, there is no evidence that this would cause long-term damage to the populations. Shore surveys did not indicate damage to intertidal flora and fauna along Fishers Island, New York, or along the southern Rhode Island coastline.

1.0 INTRODUCTION AND BACKGROUND INFORMATION

On 16 March 1978, the 166 m barge *Ocean 250,* owned by the Interstate and Ocean Transport Co. of Philadelphia, Pa., was carrying a cargo of 39.7 million liters of gasoline enroute from a British Petroleum terminal in Marcus Hook, Pa., to a Lehigh Gasoline terminal in New Haven, Conn. At 0145 h, approximately 16 km off course, the barge grounded on Watch Hill Reef (lat. 41°17.40'N, long. 71°51.50'W), 1,006 m off Watch Hill, R.I. (Fig. 1-1) Several cargo tanks ruptured and by 2250 h, when the barge lifted free of the rocks, the U.S. Coast Guard (USCG) estimated that 2.6 million liters of gasoline had been released into Block Island Sound. Because of the volatile nature of gasoline the USCG closed a 389 sq km area of Block, Fishers, and Long Island Sounds to all ship traffic. The area closed extended from Pt. Judith, R.I., to New London, Conn.

1.1 Shore Surveys

Early 16 March, University of Rhode Island (URI) personnel[2] initiated a response to the spill which included a prediction of the spill movement to determine areas that might become affected by the spill and a survey of the southern Rhode Island coastline. Overflights of the spill site were planned; however, these had to be aborted because of foul weather. Since the USCG restricted boats from entering the area, the area covered by the slick could not be surveyed and the slick was not sampled. The predicted movement of the spill was predominantly in the southeastwardly direction with the outgoing tide, and some westward movement on the incoming tide. Winds during the morning hours of 16 March were light and offshore, thereby making it very unlikely for the spill to reach the southern coastline of mainland Rhode Island.

The main concern throughout the period of the spill was the southern coastline east of Watch Hill. There is an eastward current close to shore, and if any gasoline reached shore, it could have been transported into Winnapaug, Quonochontaug, and Ninigret Ponds (Fig. 1-1). Consequently, water samples were taken at each of these inlets and at Watch Hill. Chemical analysis of the samples taken at Watch Hill and Weekapaug (inlet to Winnapaug Pond) showed no signs of gasoline. Further, marine scientists from URI and the R.I. Department of Environmental Management found no signs of impact from the spill on coastal communities from Napatree Point eastward to Pt. Judith.

Although initially the winds were from the west which drove the visible slick toward open water, by 1146 h the winds had shifted to the northeast so that the slick was driven apparently toward the eastern end of neighboring Fishers Island, N.Y., located 4.8 km WSW of Watch Hill Reef (Fig. 1-1). In order to check for the presence of gasoline and possible resulting environmental damage to intertidal flora and fauna, an onshore survey of the eastern end of Fishers Island was conducted 18 March by Jeff Hyland of the Environmental Protection Agency (South Ferry Road, Narragansett, RI 02882) and Brian Melzian of the Graduate School of Oceanography, URI (Kingston, RI 02881).

Approximately 3.2 km of shoreline along the eastern tip of Fishers Island was surveyed and photographed. The intertidal zone included sandy (with coarse gravel) substrates as well as rocky areas inhabited by typical species such as the algae *Fucus* spp., *Ascophyllum nodosum,* and *Ulothrix flacca;* the blue mussel, *Mytilus edulis;* the common periwinkle, *Littorina littorea;* and the barnacle, *Balanus balanoides.* Samples of rocky intertidal organisms were examined for adverse effects and the presence of

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[2]Mason P. Wilson, Jr., Department of Mechanical Engineering and Applied Mechanics, initiated the response and was aided by Mark Ahmadjian, Shiela Bhattacharya, Chris Brown, Gina Garofalo, Clem Griscum, Chris Ordzie, and Malcolm Spaulding, URI, and Richard Sisson, RI Department of Environmental Management. The survey information was excerpted from a letter dated March 23, 1978, by Mason Wilson to the Conservation Commission in Westerly, RI.

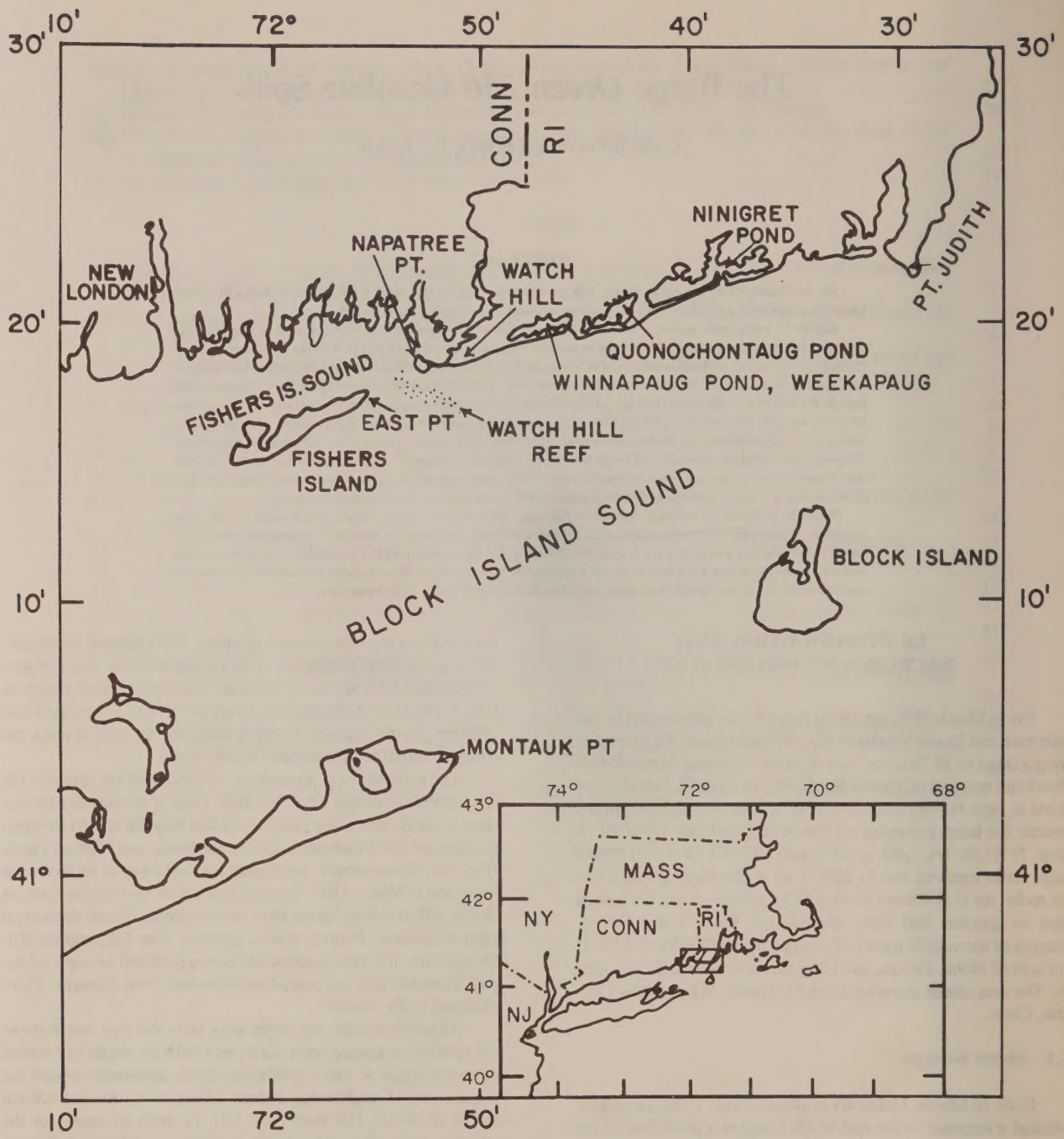


Figure 1-1.—Site of the grounding of the *Ocean 250* on Watch Hill Reef, R.I., and the immediate area including shore survey areas of Fishers Island and the southern Rhode Island coastline.

gasoline. No immediately apparent traces of gasoline (by taste or smell) or adverse biological effects (i.e., dead or moribund organisms) were noted.

No dead or moribund macroinvertebrates or fish were observed along any of the sandy beaches. Occasionally dense flotsam consisting mainly of washed-up kelp, *Laminaria saccharina,* was observed, particularly near East Point, Conn.; however, this is a normal occurrence, and there were no visible traces or smell of gasoline from these dense mats. Sandy areas were sampled every 46 m to a depth of 10 cm with a trowel to locate interstitial gasoline. Again, no traces were seen or smelled.

A large population of apparently healthy herring gulls, *Larus argentatus,* was observed. No dead or abnormally behaving birds were seen.

Although there were no visible signs or effects of gasoline, samples of sediment and *M. edulis* were collected for hydrocarbon

analysis from each of three stations established at the eastern end of the island. Samples were frozen should further interest warrant their analysis.

All the observations made on Fishers Island suggest that there was no onshore impact from the gasoline spill. Apparently a slick never reached the island. There were no dead or moribund organisms washed ashore as a result of the spill, and typical intertidal organisms were observed in a healthy state.

1.2 Damage Assessment Surveys

The National Oceanic and Atmospheric Administration (NOAA) was requested to organize the damage assessment so that on 17 March 1978, when the USCG opened the area for vessel traffic the National Marine Fisheries Service (NMFS)[3] chartered the RV *Strider* for a series of 1 d cruises on 17, 18, and 20 March 1978, from Jerusalem (17, 18) and Galilee, R.I. (20). Personnel from NMFS, the Environmental Protection Agency (EPA), and the Graduate School of Oceanography, URI, participated in these cruises. The survey was conducted over a 3.2 × 2.4 km grid off the Rhode Island coast between Watch Hill and Napatree Points (Fig. 1-2). Station locations and hydrological data are presented in Table 1-1. The same station numbers were used for all 3 d, but daily samples were distinguished by the addition of 0 to the 17 March stations, 100 to the 18 March stations, and 200 to the 20 March stations, i.e., 1, 101, and 201. For simplicity within this report most stations are referred to by the original (+0) designation.

[3]Northeast Fisheries Center Narragansett Laboratory, National Marine Fisheries Service, NOAA, South Ferry Road, Narragansett, RI 02882.

Table 1-1.—Summary of station locations and hydrologic data from RV *Strider* cruise 78-01 in the area of the grounding of the *Ocean 250*.

Station no.	Lat. (°N)	Long. (°W)	Depth (m)	Temp (°C)	Salinity (0/00)
1	41°17′02″	71°51′30″	34	1.7	29.4
2	41°17′02″	71°52′10″	34	1.7	29.4
3	41°17′02″	71°52′52″	27	1.7	29.4
4	41°17′30″	71°51′52″	5	1.8	29.0
5	41°18′02″	71°52′10″	9	2.2	28.6
6	41°18′02″	71°52′10″	6	2.2	29.5
7	41°17′30″	71°52′10″	9	1.8	29.4
8	41°17′30″	71°51′30″	21	1.7	29.9
9	41°18′01″	71°51′30″	8	1.7	29.7
10	41°17′30″	71°50′59″	26	1.7	29.5
11	41°17′30″	71°50′12″	30	1.1	30.0
12	41°17′02″	71°50′59″	37	1.1	30.8
13	41°17′02″	71°50′12″	38	1.1	31.0
14	41°18′01″	71°50′12″	29	1.1	31.0
15	41°18′32″	71°50′12″	12	1.1	31.2
16	41°18′29″	71°50′59″	7	1.4	30.5
17	41°18′01″	71°50′59″	11	1.4	29.8
18	41°18′28″	71°52′10″	5	2.2	28.8

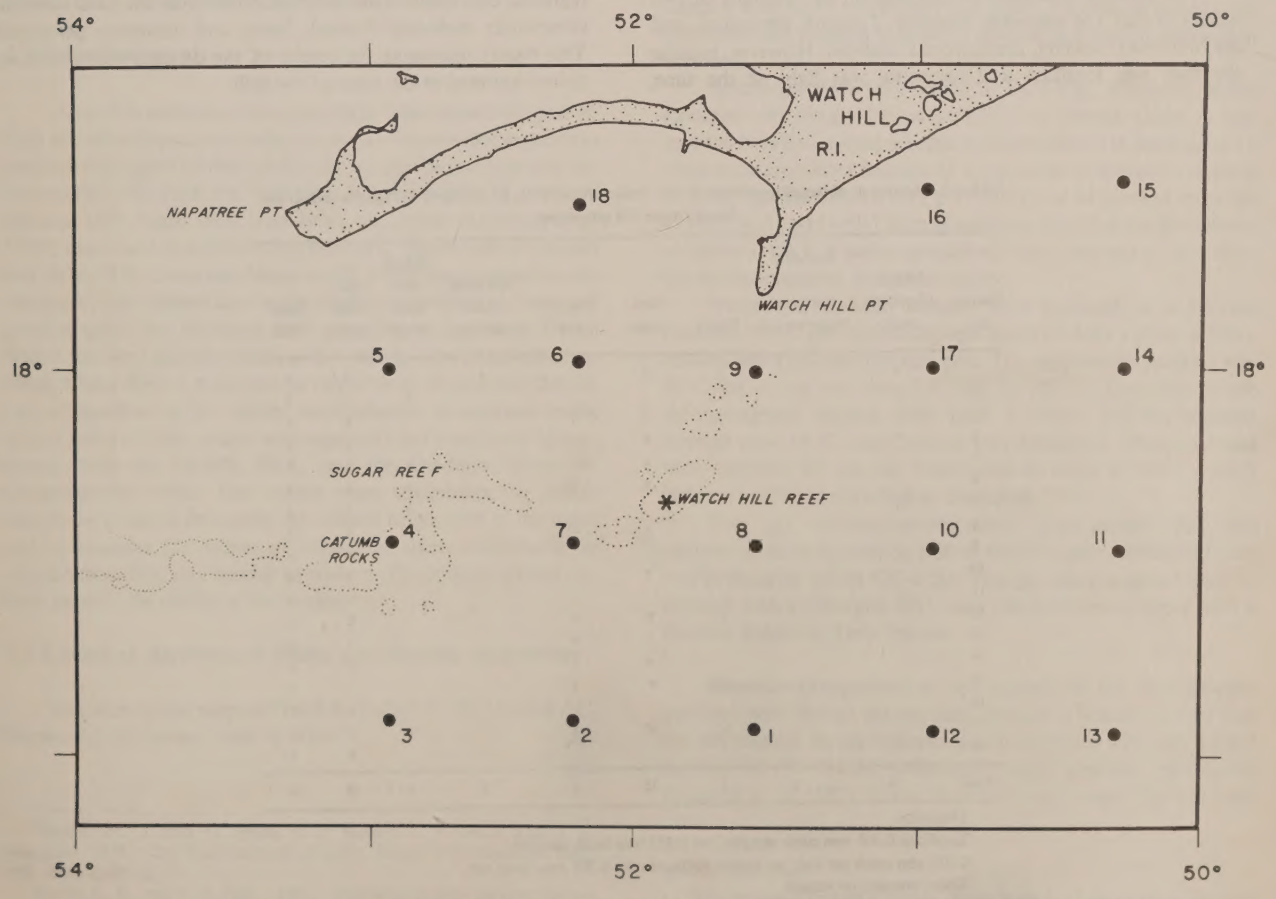


Figure 1-2.—Survey area and station locations for RV *Strider* cruise 78-01. The barge *Ocean 250* was grounded on Watch Hill Reef (*) on 16 March 1978.

A summary of samples collected for chemical and biological analyses are presented in Tables 1-2 and 1-3. Specific sampling methods and analytical procedures and techniques are discussed in the following sections on chemical and biological analyses.

The damage assessment focused on chemical analyses of gasoline hydrocarbons in the water column, fish, macroinvertebrates, and plankton; benthic and zooplankton communities structure was analyzed, and fish eggs were screened for cytogenetic damage.

1.3 Conclusions

The *Ocean 250* gasoline spill did not cause serious impact in the coastal waters off southern Rhode Island. The slick dissipated within 24 h of the spill and appeared to have been confined within a 4 km ellipse to the south and southwest of Watch Hill Reef. This is surmised from early visual observations and from shore surveys of sourthern Rhode Island and Fishers Island which revealed no traces of gasoline in the water or in the sediments.

In the area directly adjacent to Watch Hill Reef and within the sampling grid (Fig. 1-2) gasoline components were detected in the water column, in zooplankton and selected benthic invertebrates, indicating that gasoline was well mixed in the water column down to the bottom where it was accumulated either directly by filter-feeding organisms or through food chain components.

The greatest detectable impact was on the embryos of two species of fish, the yellowtail flounder, *Limanda ferruginea,* and the fourbeard rockling, *Enchelyopus cimbrius.* However, because the spill was localized and spawning was light at the time, no long-term effects on the resident populations of the southern Rhode Island coast are anticipated.

The analysis of fish flesh samples, including a control from Narragansett Bay, also indicated the presence of some gasoline components in the flesh; more important, it detected the presence of relatively high levels of other anthropomorphic hydrocarbons in many of the fish, indicating that there is a chronic release into these coastal waters. The implications of this release to renewable marine resources and public health are just beginning to be examined.

Although areas further offshore and east of the spill area have been studied in the past, there was no baseline information available against which to compare such a site specific study as this. In addition, the small sample size as well as the short sampling period do not allow for conclusions on natural variability and, therefore, it is impossible to draw definitive conclusions on the effects of the spill on species composition or abundance. However, the hydrocarbon analyses as a whole and especially those on the fish samples point out the chronic presence of anthropogenic hydrocarbons in this coastal marine environment. This, together with the deleterious effect of the gasoline on fish eggs, indicates impact, some due to the *Ocean 250* spill, some due to other sources.

The gasoline spill was the first major spill in the eastern United States since the *Argo Merchant* sank off Nantucket Shoals in December 1976. Since that time toxic spill response plans had been developed through regional workshops and individual agencies. As a result the response to the *Ocean 250* represented an integrated, coordinated, and efficient effort from the local scientific community including Federal, State, and university personnel. This report represents the results of the damage assessment activities initiated at the time of the spill.

Table 1-2.—Summary of samples collected on RV *Strider* cruise 78-01, 17-20 March 1978 in the area of the barge *Ocean 250* grounding.

Station no.	Niskin (duplicate casts)	Butterfly sterile-plastic bag water sample	Trawl	Neuston	Plankton	Rocking chair dredge	Shipek triplicate grabs	Surface temp.	Salinities
1	x		x	x[1]		x		x	x
2	x	x					x	x	x
3	x			x[1]	x[2]			x	x
4	x	x		x				x	x
5				x	x[2]	x	x	x	x
6				x	x[2]		x	x	x
7	x						x	x	x
8				x	x[2]		x	x	x
9								x	x
10			x	x				x	x
11	x							x	x
12			x	x[3]	x[3]			x	x
13				x	x			x	x
14				x	x			x	x
15			x	x[1]	x			x	x
16								x	x
17			x[4]	x[1]	x		x	x	x
18								x	x
Total	6	2	5	12	9	2	6	18	18

[1] Duplicate.
[2] Duplicate 0.505 mm mesh samples, no 0.333 mm mesh samples.
[3] 0.333 mm mesh net lost; no sample-replaced with 0.505 mm mesh net.
[4] Doors crossed; no sample.

Table 1-3.—Summary of fish and invertabrate species collected on RV *Strider* cruise 78-01 in trawls and by dredge in the area of *Ocean 250* grounding; selected species were analyzed for hydrocarbons. Crosses indicate present in sample, analyzed for hydrocarbons by Energy Resources Company; solid circles indicate present in sample, analyzed for hydrocarbons by Environmental Protection Agency; open circles indicate present in sample.

Species	Trawl stations				Fox Island control	Rocking chair dredge stations	
	10	12	15	17		1	5
Macrozoarces americanus	x	x	x	x			
Scophthalmus aquosus	x	x	x	x			
Pseudopleuronectes americanus	x	x		x	x		
Limanda ferruginea		x	x	x			
Raja erinacea	x	x		x			
Myoxocephalus octodecemspinosus		x					
Hemitripterus americanus	x						
Clupea harengus	x						
Tautogolabrus adspersus		x					
Gadus morhua		x					
Liparis sp.							
Menidia menidia							
Polinices heros							
Mercenaria mercenaria						●	
Asterias forbesi							○
Cancer irroratus							○
Placopecten magellanicus							
Modiolus modiolus						●	

2.0 CHEMICAL ANALYSES

2.1 Introduction

A need to understand the potentially deleterious ecological effects of spills of petroleum fuels has resulted in extensive research to examine the impact of these pollutants on marine and intertidal environments. Although the ecological effects of spills of crude oil (Benyon 1967; Smith 1968; Hess 1978), No. 2 fuel oil (Blumer et al. 1970[4]; Burns and Teal 1971[5]), and bunker C and #6 fuel oil (Scaratt and Sitko 1972; Gross and Mattson 1977) have been extensively investigated, only limited data are available concerning the impact of gasoline spills. The 16 March 1978 grounding of the *Ocean 250* on Watch Hill Reef and the release of 2.6 million liters of gasoline into Block Island Sound presented an opportunity to examine the impact of gasoline on the marine environments. In response to the spill, a series of joint cruises was organized and conducted by personnel from the NMFS, EPA, and the Graduate School of Oceanography, URI. The cruises were undertaken to collect samples in order to determine the content of gasoline in the water and to examine the extent of impact of these compounds on zooplankton, fish, and benthic organisms. The following three sections present the results of the studies.

2.2 Chemical Analyses of Water and Benthic Organisms

This section was prepared by J. L. Lake,[6] C. W. Dimock,[7] C. Norwood,[6] R. Bowen,[7] and B. Kyle.[7]

Sampling and analytical procedures.—Water samples were obtained on the first cruise (17 March), at the stations shown (Fig. 2-1). These 6 liter samples were obtained with a 5 liter Niskin bottle at a depth of 3.5 (±0.5) m and extracted on board with 300 ml of methylene chloride. These extracts were passed through Na_2SO_4 to remove residual water and then reduced in volume in a Kuderna-Danish evaporator fitted with a three ball Snyder reflux column. The samples were solvent exchanged to hexane, reduced in volume under a stream of nitrogen to 0.1 ml, and stored at −5°C.

Samples of filter-feeding bivalves were obtained on the second cruise (18 March) at the stations shown in Figure 2-1. Samples of horse mussel, *Modiolus modiolus*, and hard shell clam, *Mercenaria mercenaria*, were collected at station 1 with a rocking chair dredge, and sea scallop, *Placopecten magellanicus*, were collected at station 12 with a trawl. Samples were placed in plastic bags, packed in ice, and then frozen at the laboratory until analysis. Thawed samples were dissected, and the tissues digested for 16 h at 37°C in 4N NaOH in glass centrifuge tubes with Teflon-lined screw caps. The digested tissues were then extracted with methylene chloride. The organic phase was passed through a column of Na_2SO_4 and silica gel to remove interfering material, and subsequently reduced in volume and solvent exchanged to hexane. Selected tissue samples were separated on a second column of silica gel into an aliphatic and an aromatic hydrocarbon fraction. All fractions were reduced in volume to 0.1 ml under a stream of nitrogen and stored at −5°.

To prevent an excessive loss of the more volatile, low molecular weight components of the gasoline, the extracts of the samples were never evaporated to dryness. Procedural blanks were processed with samples in order to determine if contamination had occurred.

Several gasolines were spilled by the barge. Standards of the gasolines were obtained from the USCG at Groton, Conn. A neat gasoline standard, a neat gasoline standard diluted in hexane, and a water-accommodated fraction of the gasoline (prepared by shaking 10 ml of gasoline from the #1 port and 10 ml of gasoline from the #1 starboard tanks with 2 liters of seawater, and allowing the mixture to separate for 6 h before extraction) were analyzed to determine the efficiency of the analytical method.

Water and shellfish samples were analyzed in a Hewlett Packard 5840A gas chromatograph equipped with a glass capillary column and a splitless injection port. The temperature program was 35°C for 4 min and then 5°C/min to 250°C. The areas of the chromatograms ranging from peak 1 (Table 2-1) (C_2-benzene) through peak 15 (C_2-naphthalene) were integrated. Quantifications were obtained by relating these areas to areas of known alkyl benzene and alkyl naphthalene standards.

The gas chromatographic-mass spectrometric (GC-MS) analyses were performed on a 30 m OV-101 glass capillary column in a Schimazdu model GC-4CM. This gas chromatograph was interfaced with a Finnegan 1015 mass spectrometer equipped with a Systems Industries Data System.

Results.—Comparison of the analysis of the neat gasoline standard with that of the standard diluted in hexane showed that the solvent peak on gas chromatograms interfered with only a small number of the lowest molecular weight gasoline compounds (Dimock et al. 1980). Peaks in procedural blanks (Fig 2-2) were

[4]Blumer, M., J. Sass, G. Souza, H. L. Sanders, J. F. Gassle, and G. R. Hampson. 1970. The West Falmouth oil spill. Woods Hole Oceanogr. Inst., Tech. Rep. 70-44, 32 p.

[5]Burns, K. A., and J. M. Teal. 1971. Hydrocarbon incorporation into the salt marsh ecosystem from the West Falmouth oil spill. Woods Hole Oceanogr. Inst., Tech. Rep. 71-69, 24 p.

[6]Environmental Protection Agency, South Ferry Road, Narragansett, RI 02882.

[7]Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881.

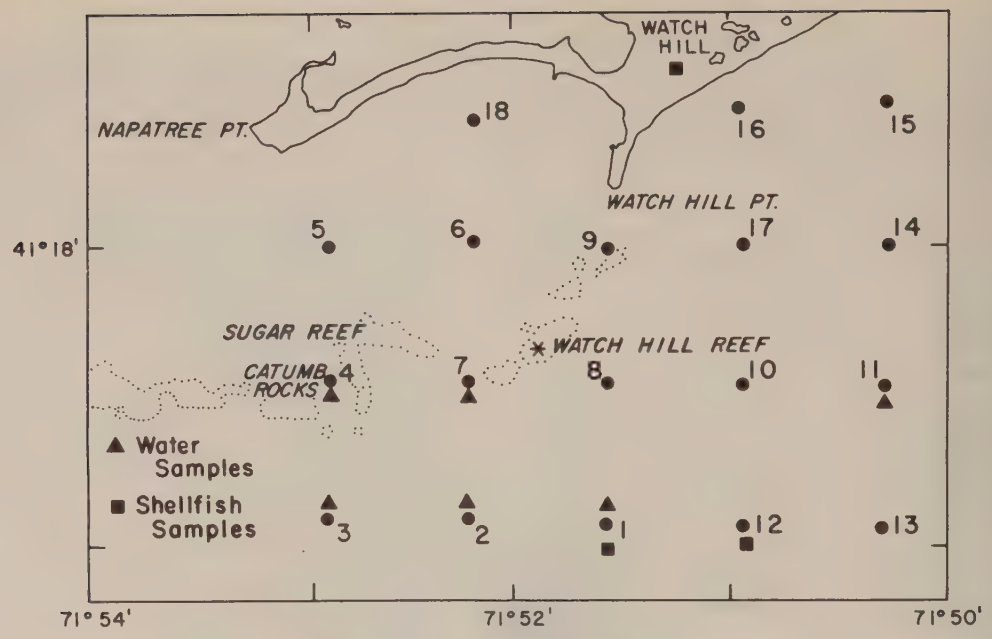


Figure 2-1.—Station sites at which water samples and bivalves were collected during cruises on 17 and 18 March 1978. The *Ocean 250* was grounded on Watch Hill Reef.

Table 2-1.—Components of the *Ocean 250* gasoline tentatively identified by mass spectrometry.

Peak number	Molecular weight	Compound
1	106	C_2-benzene
2	106	C_2-benzene
3	106	C_2-benzene
4	120	C_2-benezene
5	120	C_3-benzene
6	120	C_3-benzene
7	120	C_3-benzene
8	120	C_3-benzene
9	120	C_3-benzene
10	118	Indane
11	134	C_4-benzene
12	128	Naphthalene
13	142	2-methyl naphthalene
14	142	1-methyl naphthalene
15	156	C_2-naphthalene

small and interfered only slightly with analyses of the higher molecular weight gasoline compounds. A comparison of the analyses of the gasoline standard diluted in hexane with that of the water-accommodated fraction of the gasoline taken through the analytical procedure showed approximately 70% of the higher molecular weight compounds (i.e., C_2-naphthalenes) and approximately 50% of the lower molecular weight compounds (i.e., C_2-benzenes) were returned. Average recovery for all compounds was approximately 60%. The analyses of water samples in this report, however, were not corrected for these losses in the analytical procedure.

Gas chromatograms of extracts from the water samples from stations 3, 4, and 7 revealed a pattern of peaks that was qualitatively similar to the patterns from the water-accommodated fraction and the gasoline standards. The water-accommodated fraction, the gasoline standard, and extracts from the station 7 water sample are compared in Figure 2-3. There was, however, an apparent greater relative loss of some of the lower molecular weight compounds in the water samples. Gas chromatograms of water samples from stations 1 and 11 showed no detectable gasoline compounds, and the sample from station 2 showed only trace levels (Table 2-2). GC-MS analyses of the compounds in water samples from stations 3, 4, and 7 showed that these compounds were alkylated benzenes, indane, naphthalene, and alkylated naphthalenes (Table 2-1).

Table 2-2.—Concentrations of gasoline range hydrocarbons in water samples taken off Watch Hill Reef (*Ocean 250* spill). Concentrations were not corrected for losses during extraction or chromatographic procedure.

Water station no.	Total gasoline hydrocarbons (C_2-benzenes—C_2-naphthalenes)
1	Background value
2	Trace levels (1.0 ppb)
3	3 ppb
4	10 ppb
7	12 ppb
11	Background value

Extracts of shellfish showed gas chromatographic patterns similar to those obtained from gasoline standards in most samples (Fig 2-4), but interfering peaks were also prominent. GC-MS analyses showed many identical spectra from peaks in extracts of the *Mercenaria mercenaria* sample and from the gasoline standard.

Discussion.—Experiments conducted to evaluate the analytical method showed it could consistently recover and measure most of the gasoline present in water samples (Dimock et al. 1980). The solvent peak on gas chromatograms obscured only a small number of gasoline peaks, and procedural blanks showed that interfering material was not introduced by the analysis. The average efficiency of recovery for the procedure was 60%, although it was more efficient in recovering the less volatile, higher molecular weight com-

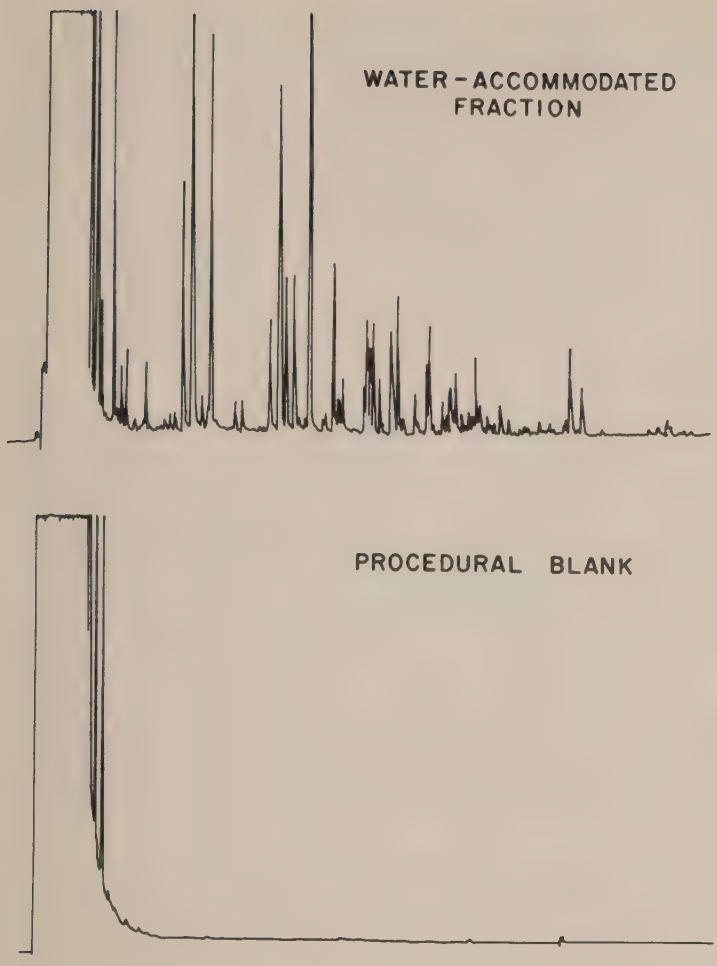


Figure 2-2.—Gas chromatograms of the water-accommodated fraction of gasoline and a procedural blank. Both analyses were run on a 30 m SE-52 glass capillary column: 35°C for 4 min, the 5°/min to 250°C.

pounds (70%) than the lower molecular weight compounds (50%). This relatively greater loss of low end components was found to occur during the final volume reduction phase of analyses.

While visible slicks and gasoline odors were not apparent when the water samples were taken, comparisons of gas chromatograms from gasoline standards with those from water samples from stations 3, 4, and 7 clearly indicated the presence of gasoline compounds (for station 7, see Figure 2-3). GC-MS analysis corroborated the presence of gasoline by identifying alkylated benzenes, indane, naphthalene, and alkylated naphthalenes in extracts from these water samples. The levels of gasoline in these samples ranged from 3 to 12 ppb, with only trace amounts present in the sample from station 2. These stations were southwest of Watch Hill Reef which was the direction the slick moved before dissipating (Fig. 2-1). Samples from station 1, to the south of Watch Hill Reef, and from control station 11, to the east, did not show detectable levels of gasoline compounds. When compared with the gasoline standards and the water-accommodated fraction, chromatograms of water samples showed relative decreases in the amounts of lower molecular weight gasoline compounds (Fig. 2-3). While these decreases were at least partly due to losses in the final volume reduction during analysis, they may also reflect greater volatilization of the low molecular weight compounds to the atmosphere during environmental exposure.

With the exception of the sea scallop adductor muscle, which showed very low levels of compounds on gas chromatograms, samples of shellfish tissues showed low levels of compounds in the molecular weight region of gasoline. Comparisons of gas chromatograms of a water-accommodated gasoline standard with those from shellfish suggested the presence of gasoline compounds in these tissue samples (Fig. 2-4). The correlation of peak distribution patterns in gas chromatograms from the gasoline standards and the bivalve tissues was not as clear as with the water samples because of the low levels found in the tissues, and due to interfering biogenic material. However, GC-MS analysis of tissues of the *Mercenaria mercenaria* samples showed the presence of hydrocarbon compounds typical of gasoline. Comparison with gas chromatograms from other bivalve samples suggested that they also may have contained these gasoline compounds. Since control organisms sampled prior to the spill were not available, it was not possible to determine if the presence of these compounds in the organisms resulted from the spillage of gasoline from the *Ocean 250* or from other sources such as industrial and municipal wastes.

The accumulation and depuration of hydrocarbons from petroleum fuels by filter-feeding bivalves has been investigated (Blumer et al. 1970; Lee et al. 1972; Stegeman and Teal 1973; DiSalvo et al. 1975; Boehm and Quinn 1977; among others). In general these studies found that the majority of accumulated petroleum hydrocarbons were rapidly depurated from the organisms; however, Blumer et al. (1970) with *Crassostrea virginica*, and Boehm and Quinn (1977) with *Mercenaria mercenaria* found slow depuration of accumulated petroleum hydrocarbon com-

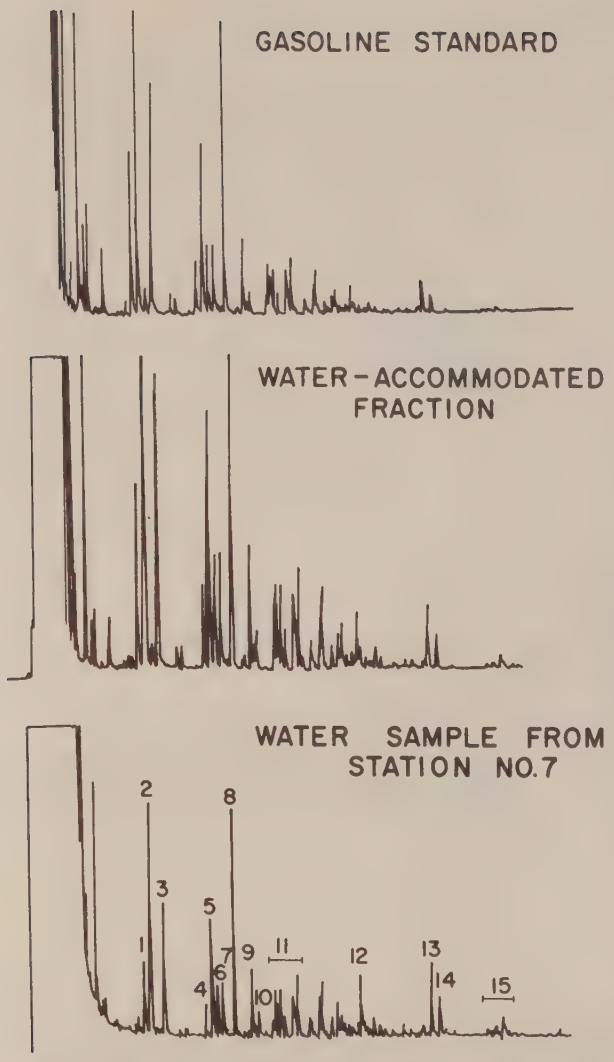


Figure 2-3.—Gas chromatograms of a gasoline standard in hexane, a water-accommodated fraction (WAF) of the gasoline standard, and a water sample from station 7. The standards were run on a 30 m SE-52 glass capillary column. The water sample was run on a 30 m OV-101 column. All analyses: 35°C for 4 min, the 5°/min to 250°C.

pounds. Obviously, if the gasoline compounds found in filter-feeding bivalves in the present study were retained for extended periods, they would have a greater potential to harm the organisms and the consumers (including man).

The toxicity of gasoline and its individual components to marine organisms has been investigated through several laboratory research efforts (Meinck et al. 1965; Crapp 1971; Brocksen and Bailey 1973; Jacobsen and Baylou 1973; Yevich and Barszcz 1976), but not much information is available concerning the envinronmental impact of spills of gasoline. Bugbee and Walter (1973) conducted a biological survey along 21 km (13 mi) of Grace Coolidge Creek (South Dakota) where 18.9 thousand liters (5,000 gal) of aviation gasoline were spilled in November 1969. They found the biological impact to be severe, and almost a full year was required for nearly complete recovery. Because of the difficulties of extrapolating laboratory toxicity tests to field situations and due to the paucity of information concerning the impact of gasoline spills in the environment, it is difficult to assess, or even estimate, the ecological damage inflicted by the *Ocean 250* spill. Nevertheless, it is apparent from this study that gasoline from the *Ocean 250* did penetrate the marine water colmn in the vicinity of Watch Hill Reef. Gasoline was found in several water samples, and the data suggest that gasoline from the accident may have been accumulated by organisms at a depth of up to 10 m.

Acknowledgment.—The assistance of funding from the EPA Grant No. R805477-02 is gratefully acknowledged.

2.3 Hydrocarbon Analyses of Plankton Samples

This section was prepared by E. J. Hoffman[8] and J. G. Quinn.[8]

Collection procedures.—Plankton tows were conducted aboard RV *Strider* on 20 March 1978 (4 d after the *Ocean 250* gasoline spill) within an area 3.2 × 2.4 km between Watch Hill Point and Napatree Point, R.I. The station locations and location of the grounding along with the gasoline hydrocarbon analysis results of the water samples are given in Figure 2-5. The planktons were collected with paired 61 cm diameter bongo frames fitted with 0.505 mm and 0.333 mm mesh nets towed in continuous double oblique patterns for 15 min. The 0.333 mm mesh sample was split; half of which was preserved for biological studies and the other half, used for the hydrocarbon studies, was frozen until analyzed. The 0.333 mm net was lost after four stations and the frame was refitted with a 0.505 mm net. Thereafter, one 0.505 mm net sample was split in the above manner and saved for hydrocarbon analyses. The collection data is given in Table 2-3.

Analytical procedure.—The plankton samples were thawed and the seawater associated with the samples was removed by passing the sample through a 45 mm stainless steel sieve. The plankton retained by the sieve were transferred to tared 50 ml centrifuge tubes. The samples were not dried in order to prevent loss of volatile gasoline components. (An estimate of the percentage moisture was determined on three separate plankton samples collected later especially for this purpose.)

[8]Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881.

Table 2-3.—Plankton samples-collection data, *Ocean 250* spill, 20 March 1978.

Station at start of tow[1]	Station at end of tow	Time of start	Time of end	Mesh size used for HC analysis split
213	211	0937	0952	0.333
214	215	0956	1011	0.333
215	216	1023	1038	0.333
217	210	1050	1105	0.333
212	201	1114	1129	[2]0.333
208	209	1153	1207	0.505
206	218	1221	1236	0.505
205	204	1244	1259	0.505
203	202	1309	1324	0.505

[1]Station numbering system is organized as follows: The prefix of two preceeding two digit station number refers to samples collected on the 3d leg of RV *Strider* 78-01. For example, 213 refers to a sample collected on 20 March 1978 (3d leg) at station 13. (The text refers to the station at the beginning of the tow).

[2]Sample lost during collection.

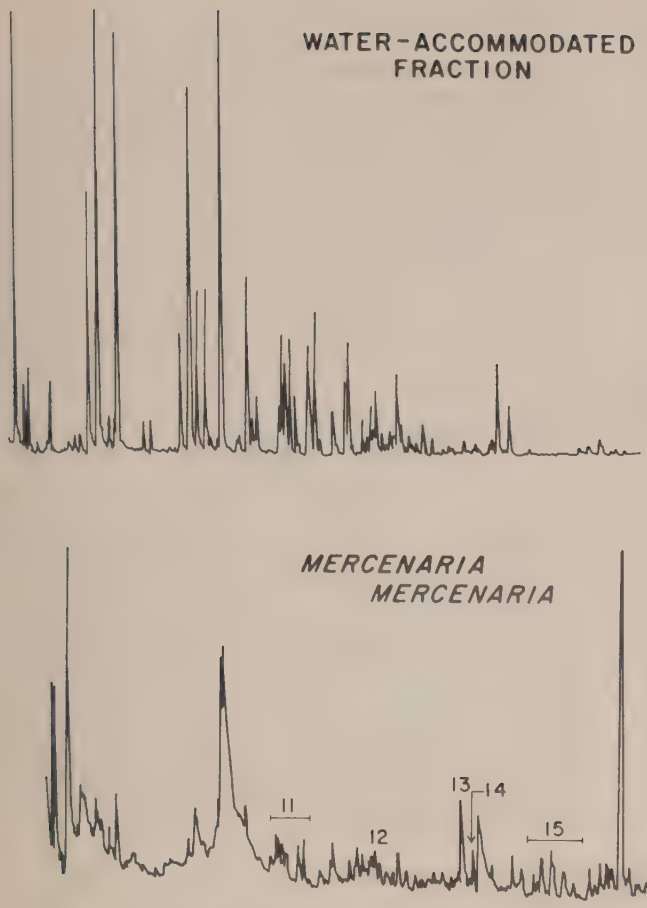


Figure 2-4.—Gas chromatograms of the water-accommodated fraction (WAF) of the gasoline and of *Mercenaria mercenaria* from station 1. The WAF was run on a 30 m SE-52 glass capillary column. *Mercenaria mercenaria* was run on a 30 m 30 m SE-54 column. Both analyses: 35°C for 4 min, the 5°/min to 250°C.

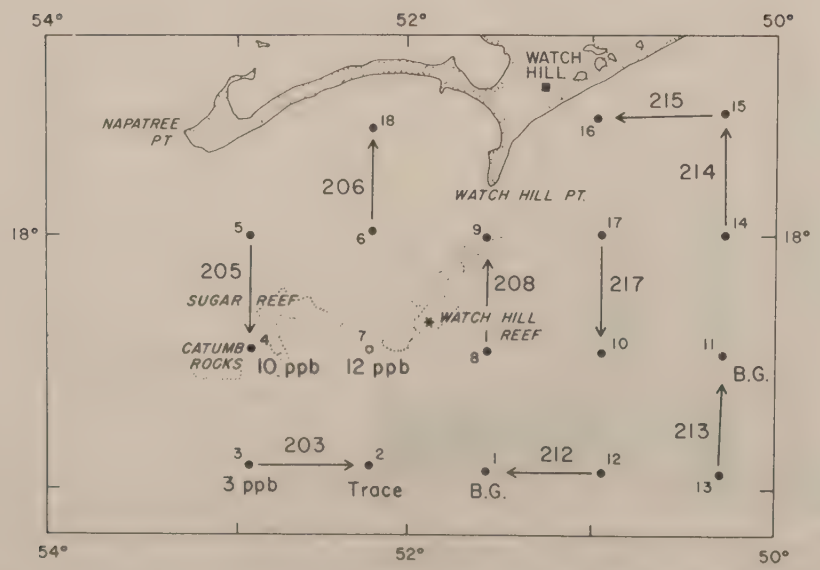


Figure 2-5.—Station locations, zooplankton tow directions, and water column gasoline hydrocarbon values at *Ocean 250* spill site.

Each wet plankton sample was saponified (under nitrogen) with 0.5 N KOH-methanol in tightly capped centrifuge tubes standing in a boiling water bath for 30 min. The resulting mixtures were cooled and the hydrocarbons (with nonsaponifiable lipids) were extracted three times with 10 ml portions of hexane. The hexane extracts were evaporated to 0.5 ml on a rotary evaporator at 30°C. One μl of this extract was injected into a Hewlett Packard 5840A gas chromatograph equipped with a 15 m OV-101 glass capillary column. The injection and programming conditions are given in Table 2-4.

Three types of standards were used in this study. For quantification, a measured amount of a mixed gasoline standard (1:1:1:1:1, Tank 1 Starboard, Tank 1 Port, Tank 2 Port, Tank 3 Port, and Tank 4 Port) was carried through the entire saponification-extraction procedure. The hydrocarbon activity of this process standard was limited to peaks between retention times (RT) of 0 and 25 min, boiling range about $\sim C_6$-C_{14} (see Fig. 2-6). The areas under each peak in the plankton sample chromatogram from RT = 7 to 25 min were summed. The area was related to concentration by use of a calibration curve constructed using various injection volumes of the processed standard. It was necessary to construct a calibration curve using this gasoline standard because the relationship between area and concentration was not linear and did not intercept zero. The two other standards, a nonprocessed gasoline mixture and a water-accommodated gasoline standard were used for matching purposes employing the procedure of Hoffman and Quinn (1978).

Table 2-4.—Glass capillary analytical conditions, *Ocean 250* spill.

Volume injected	1 μ l
Column	glass capillary, OV-101, 15 m, 0.25 mm i.d.
Column flow	1.7 ml/min
Auxiliary flow	21.5 ml/min
Programming	Temperature 1 = 35°C, held for 5 min, then the temperature was increased at a rate of 4°/min up to 250°C
Instrumentation	Hewlett Packard 5840A gas chromatograph with integrator

Results and discussion.—The quantitative results for the plankton samples are given in Table 2-5. While each plankton sample had readily measurable amounts of hydrocarbons, presumable natural and anthropogenic sources, only one sample (205) had hydrocarbon activity in the boiling range (C_6-C_{14}) of the gasoline components. A comparison of the plankton sample, having gasoline components with one example of plankton with little or none of these components, is illustrated by chromatograms in Figure 2-7. The values given in Table 2-5 do not reflect total hydrocarbons. Total hydrocarbons for open ocean plankton samples have been previously shown to be on the order of 1-6% of the total lipid material (Lee et al. 1971). On a dry weight basis, this would represent 2,000 μg/g. Total hydrocarbons in Georges Bank population have been reported in the range between 54 g/g and 11,000 g/g (Boehm 1977[9]). The detection limits are also given in Table 2-5. Due to the high concentration of total hydrocarbons in each sample, it was not feasible to inject larger amounts into the chromatograph in order to attempt to see lower concentration levels of gasoline components without overloading the column severely with the higher molecular weight fraction. One persistent problem which affects all plankton analyses results from passing large volumes of water through a net to collect the plankton. Adsorption of organic material to the net and to particle surfaces during collection may also result in the collection of hydrocarbons from the water as well as hydrocarbons associated with the plankton. Since previous studies have reported net fouling after oil spills (Gross and Mattson 1977), it is uncertain whether the high value found in plankton sample 205 was associated with the plankton or was collected from the water column.

[9]Boehm, P. D. 1977. Hydrocarbon chemistry. *In* New England Environmental Benchmark, Fourth Quarterly Summary Report, October 13, 1977, Energy Resources Co. (BLM Contract AA 550-CT6-51), Chapter 5.1. Energy Resources Co., 185 Alewife Parkway, Cambridge, MA 02138.

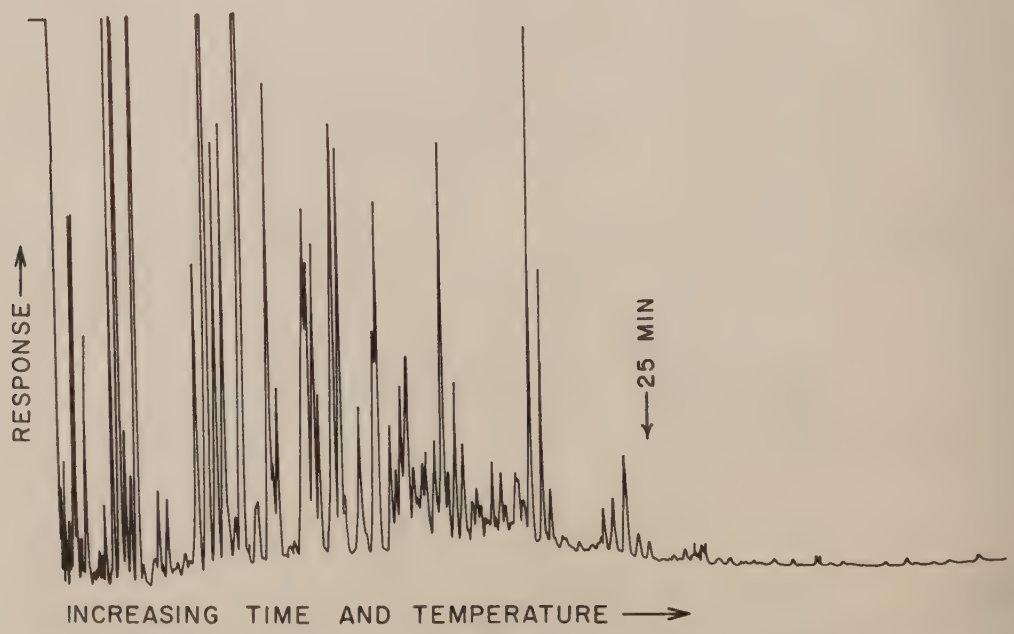


Figure 2-6.—Chromatogram of gasoline mixture from the *Ocean 250* spill carried through saponification-extraction procedure.

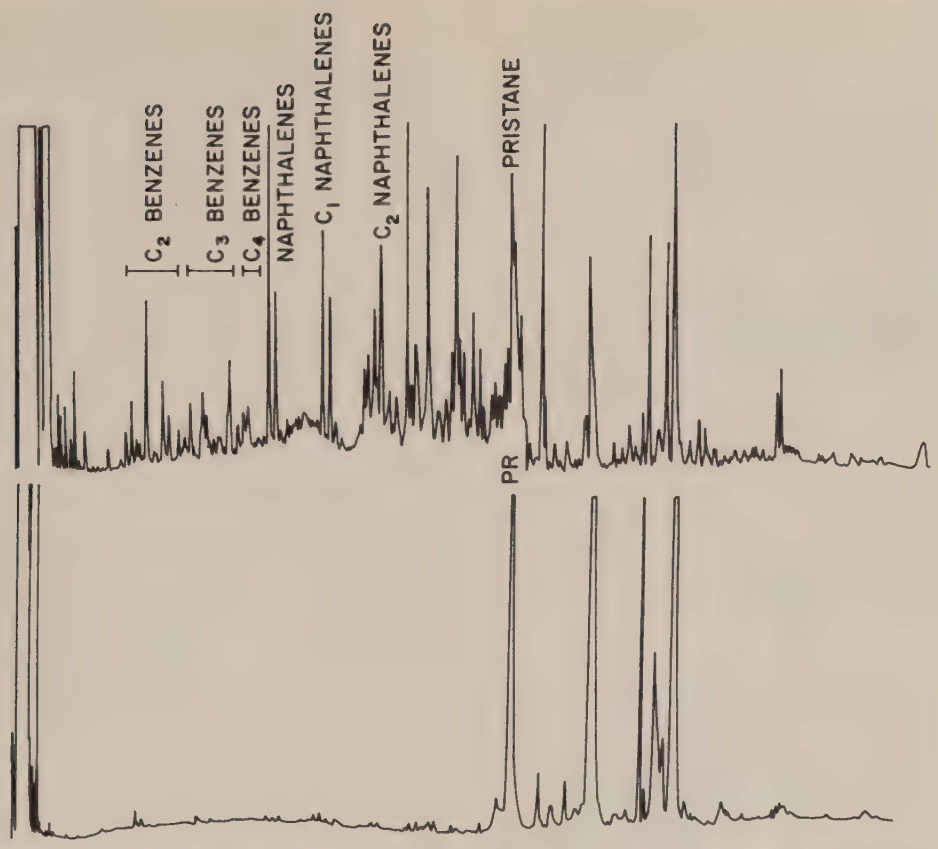


Figure 2-7.—Contrasting chromatograms from the *Ocean 250* spill. Upper, sample 205 indicates presence of gasoline components; lower, little or no gasoline components are present in sample 213.

Table 2-5.—Concentration of gasoline range hydrocarbons in plankton samples collected near the site of the *Ocean 250* grounding.

Sample	Weight of sample (wet wt.) g	Weight of sample[1] (dry wt.) g	Concentration of gasoline[2] hydrocarbons (μh HC/g wet wt.)	Concentration of gasoline[2] hydrocarbons (μg HC/g dry wt.)
203	3.44	0.276	< 88	<1,100
205	1.80	0.144	475	6,000
206	3.75	0.301	<128	<1,600
208	2.62	0.201	< 61	< 760
213	11.46	0.920	< 20	< 250
214	3.96	0.318	< 58	< 720
215	6.08	0.488	< 38	< 470
217	7.91	0.635	< 29	< 360

[1]Calculated from three plankton samples collected on RV *Strider* 78-02; average moisture content was 92 ± 1%.

[2]Boiling range ~ C_6-C_{14}.

Qualitatively, the plankton sample 205 had 18 peaks in common with the water-accommodated gasoline chromatogram (Fig. 2-8). In order to examine the match of the sample hydrocarbons with the hydrocarbons in the gasoline, the matching technique of Hoffman and Quinn (1978) which plots individual peak areas of the sample chromatogram versus the individual areas of corresponding peaks of a standard was used in this study. The matching exercise in this case, however, was complicated by the wide assortment of standards available. The three standards used in the course of these experiments were as follows: 1) a 1:1:1:1:1 mixture of gasoline samples from each of 5 tanks; 2) water-accommodated gasoline sample; and 3) 0.1 ml of the gasoline mixture carried through the saponification-extraction procedure used in the plankton analyses. Hereafter, these standards will be called standard 1, standard 2, and standard 3.

Direct comparison of the plankton sample 205 with standard 2 (water-accommodated gasoline) and standard 3 (processed gasoline) yielded very low correlation coefficients of 0.02 and 0.12. However, a comparison of standard 1 and standard 3 revealed a loss of the more volatile components of the whole gas (standard 1) when carried through the analysis procedure (standard 3). Figure 2-9 illustrates the nature of these routine losses. When the zooplankton sample 205 was corrected for these losses, the comparison of the sample with standard 2 yielded an improved correlation coefficient of 0.74. It is, therefore, clear that evaporative losses of hydrocarbons from the zooplankton sample, either in the environment or in the analysis, was responsible in some degree for the lack of a correlation when the sample was matched without these corrections.

A comparison of standard 2 and standard 3 yielded a correlation coefficient of 0.97, indicating that the losses occurring during processing also occurred in the process of water accommodation by evaporation or selective solubilization. If these losses occur in the laboratory during the 6 h of standing needed to prepare the water-accommodated standard (J. Lake[10]), it is reasonable to assume that

[10]James Lake, Environmental Scientist, Environmental Protection Agency, South Ferry Road, Narragansett, RI 02882, pers. commun. March 1978.

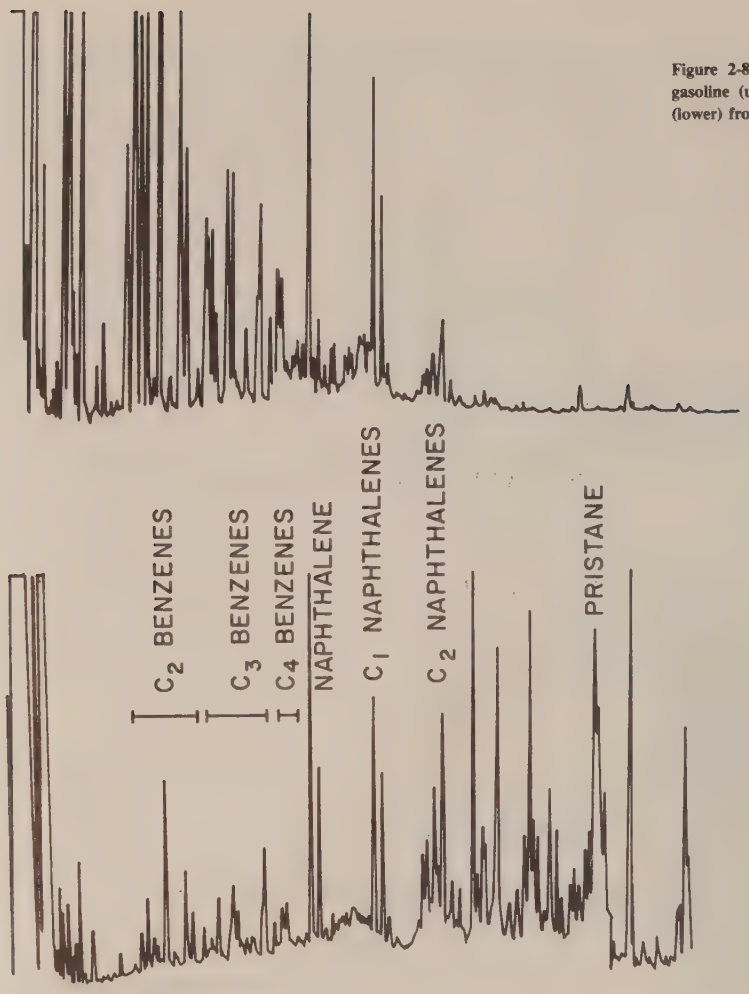


Figure 2-8.—Correspondence between chromatogram of water-accommodated gasoline (upper) and lower molecular weight portion of plankton sample 205 (lower) from the *Ocean 250* spill.

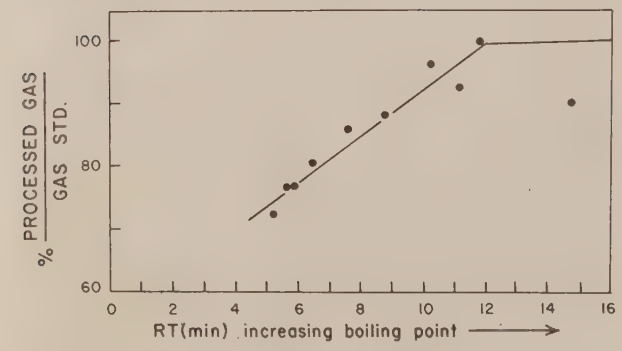


Figure 2-9.—Recovery of volatile gasoline components in saponification-extraction procedure. (RT = retention time.)

such losses are also occurring in the environment, especially considering the wave action, and the lapse of 4 d of time between the spill and the plankton collection. In addition to evaporative losses in the environment, the plankton may also selectively incorporate, metabolize, or retain only certain portions of the water-accommodated gasoline or gasoline droplets. A comparison of panels in Figure 2-8 shows that, while the alkyl benzenes are the most concentrated hydrocarbons in the standard, the naphthalene and substituted naphthalenes were the most concentrated in the plankton. Whether this is due to evaporative effects or selective uptake is unknown.

While there was not a good match in a quantitative sense between the plankton sample 205 and the gasoline standards presumably due to evaporative losses, selective solubilization, and/or selective uptake, there are two circumstantial considerations which suggest that the gasoline-type components in plankton sample 205 were from the *Ocean 250* gasoline spill: 1) There were 18 different hydrocarbons in the gasoline which were also found in this plankton sample; 2) this sample was collected near stations which had measurable amounts of gasoline components in water samples which were collected 2 d before the plankton samples (3-12 ppb gasoline hydrocarbons; J. Lake, footnote 10).

Acknowledgments.—The laboratory analyses were supported by EPA grant R805477, and the ship time was funded by NOAA.

We wish to thank Carolyn Griswold, NMFS, for the sample collection and data collection; James Lake and Barbara Kyle, EPA, for the water-accommodated gasoline standard; the USCG for the samples of gasoline from the barge; and Kurt Norwood for the GSMS identification of aromatic components in the gasoline.

2.4 Chemical Analyses of Fish Samples

This section was prepared by P. D. Boehm[11] and J. E. Barak.[11]

Collection procedures.—A total of five 30 min groundfish trawls were made on 18 and 20 March 1978, using a 12 m net (Table 1-2). The doors crossed on one trawl so no sample was obtained. Fish and invertebrates were sorted at each station, identified to species, and were frozen for hydrocarbon analyses (Table 1-3).

Analytical procedure.—The fish were in good condition and remained frozen until tissue preparation was begun. Each individual fish was filleted and the sample obtained from a combination of the skin, adipose tissue, and muscle. The samples were cut into small pieces and wet weight (25-125 g) obtained (a subsample was kept for dry weight determinations). The weight range was due to both the size of the specimens and the number of individuals comprising each sample. The tissues were then digested in 0.3 N KOH-methanol in a screw-capped flask. The digestion mixture was covered with a layer of 25 ml pentane (Resi-Analyzed, Baker). The tissues were digested for 48 h on a shaker table after which time the mixture was extracted three times with pentane. The combined pentane extracts were concentrated down to 0.5 ml, and this volume was passed through a clean-up column of fully activated silica gel to remove polar lipid material. After eluting the column with 5 ml pentane, the eluate was concentrated under purified nitrogen to 50 μl.

One microliter out of 50 was injected into a Hewlett Packard 5840A gas chromatograph equipped with a spitless injector system linked to a 15 m SE-30 glass capillary (WCOT) column. The oven was held at 30°C for 15 min and then temperature programmed at 2°C/min to an upper temperature of 260°C. Peaks in the gasoline range were integrated directly by the gas chromatographic microprocesser.

Concentrations were determined by comparison of the total area of the sample peaks in the gasoline range to that in a tissue spike of the combined cargo (Fig. 2-10). One microliter of a combination of the five cargos was spiked to fish tissue, and the spiked sample was carried through the entire procedure. Recovery of the spike was 95% relative to a direct injection of the cargo mixture on the gas chromatograph. To check on the possibility of evaporative losses in the sample concentration (evaporation) steps, a spike was administered to 250 ml $CHCl_2$ and the spikes solvent evaporated to 50 μl, first by rotary evaporation and finally by careful use of a purified nitrogen stream. Losses by evaporative concentration were negligible. Therefore, the quantification procedure which was based on the detector response of an injection of a known volume of the recovered cargo spike was a valid and reproducible method.

Results.—The gas chromatogram of the spiked fish tissue (Fig. 2-10) illustrates those components whose quantifications were the analytical objective of this study. All samples contained some higher molecular weight (HMW) hydrocarbon material. This is especially evident in the control sample (Fig. 2-10) and in other samples so designated in Table 2-6. The chemical nature of this con-

[11]Energy Resources Company, Inc., 185 Alewife Brook Parkway, Cambridge, MA 02138.

Table 2-6.—Data summary of hydrocarbon analysis of fish samples collected near the site of the *Ocean 250* grounding. Samples designated G had at least twice the control levels of measured components in the gasoline range. Those samples designated HWM had higher molecular weight petroleum components.

Sample	Station	Days after spill	Concentration of components in gasoline range from Figure 2-10 (mg/g dry weight of tissue)	Designation
Macrozoarces americanus	12	+2	0.4	
Scophthalmus aquosus	12		0.5	
Pseudopleuronectes americanus	12		2.4	G
Limanda ferruginea	12		1.9	G
Raja erinacea	12		0.2	
Myoxocephalus octodecemspinosus	12		0.5	
Tautogolabrus adspersus	12		0.6	
Gadus morhua	12		1.1	
Macrozoarces americanus	15		1.7	G
Scophthalmus aquosus	15		0.6	HMW
Limanda ferruginea	15		0.6	HMW
Macrozoarces americanus	1	+4	1.6	G
Scophthalmus aquosus	1		0.5	
Pseudopleuronectes americanus	1		0.5	
Limanda ferruginea	1		0.3	HMW
Raja erinacea	1		1.1	
Macrozoarces americanus	10		1.0	
Scophthalmus squosus	10		0.2	
Pseudopleuronectes americanus	10		0.7	
Raja erinacea	10		0.2	
Hemitripterus americanus	10		1.3	
Clupea harengus	10		1.9	G
Pseudopleuronectes americanus Control			0.7	HMW
Blank			0.0	

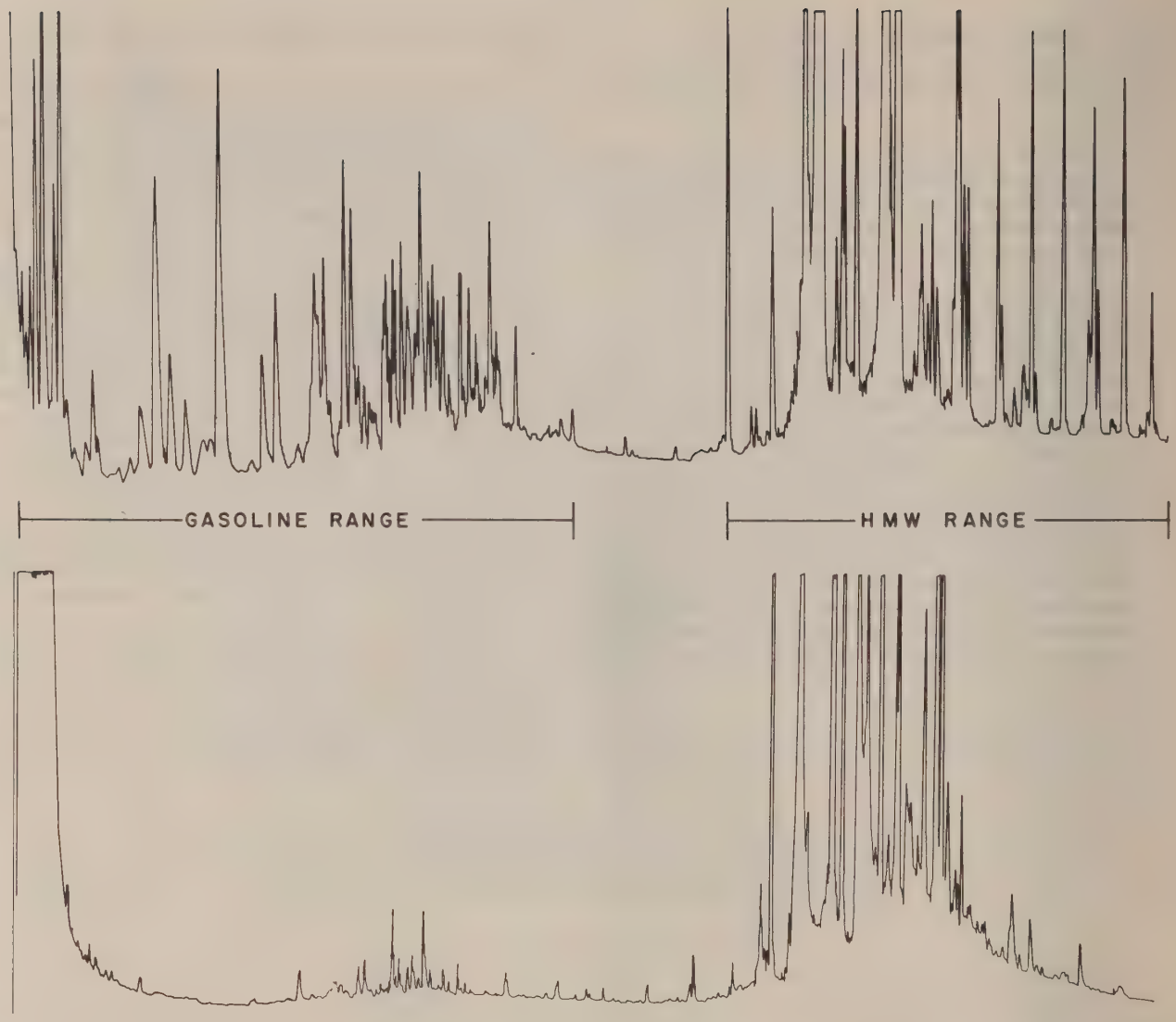


Figure 2-10.—A sample spiked with *Ocean 250* gasoline to illustrate two ranges of interest on the traces, that for gasoline and that for higher molecular weight (HMW) petroleum and biogenic hydrocarbons. This can be compared to the lower gas chromatographic trace in which is illustrated a small amount of gasoline components and a larger amount of HMW *biogenic* compounds found in yellowtail flounder collected at Station 112 in the area of the *Ocean 250* spill.

trol sample is of crucial importance in evaluating the analytical results of the other samples. As Figure 2-10 illustrates, there are several components in the gasoline boiling range (probably alkyl benzenes) present in the control animals. The chemical nature of these components is unknown at present. Examination of their structure and identification by combined GC-MS is suggested for further study. At present their structure and origin remains uncertain. Many of the actual samples contain these components, but it is difficult to ascribe their presence to recent incorporation of gasoline due to their presence in the control.

However, Table 2-6 represents an attempt at an objective quantification of these compounds. Based on 1) the qualitative scrutiny of the gas chromatograms and 2) quantitative values higher than the control sample, five of the samples *may* contain small amounts of gasoline components acquired from the spilled gasoline. Without statistically rigorous measurements of control levels of these components, we cannot be sure of a cause and effect relationship concerning the component levels associated with those samples designated by "G" in Table 2-6. The "G" designations in Table 2-6 reflect those samples having at least twice the control levels of the measured components.

Of perhaps equal interest is the presence of large quantities of HMW petroleum compounds in four of the flounder samples, including the control (Figs. 2-11, 2-12). The homologous series of n-alkanes and the dominance of the unresolved "hump" are keys to the HMW designation. Although not quantified, levels of HMW are *two orders or magnitude* (10^2) higher than the gasoline concentrations observed. The possibility remains that the fish specimens had depurated any assimilated gasoline during the 2-4 d period between the spill and when the fish were captured and frozen.

Conclusions.—The processes of selective solubilization and selective uptake of gasoline components complicates our ability to present definitive "total gasoline concentrations." A more fruitful approach would be to focus on several key components—perhaps alkyl benzenes—and to follow their incorporation into marine

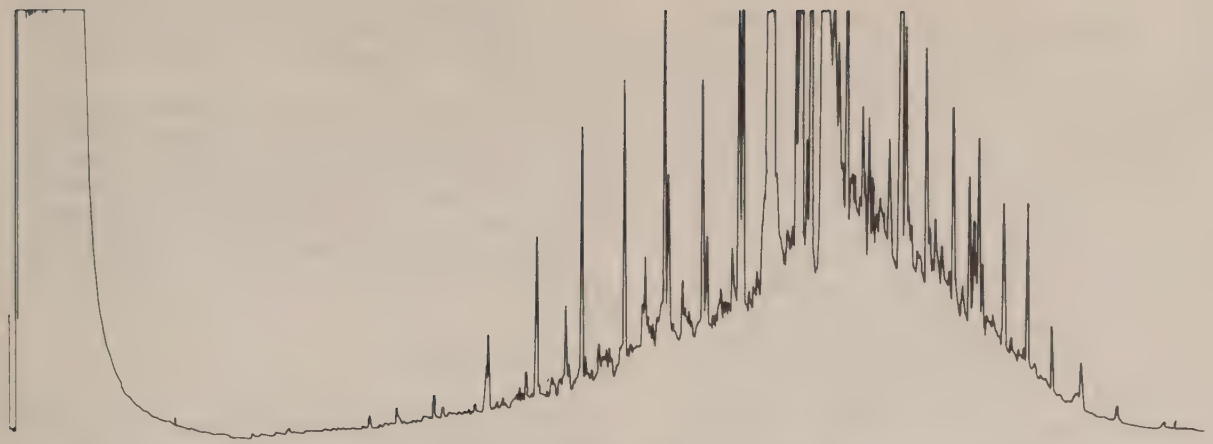


Figure 2-11.—Gas chromatographic trace illustrating no gasoline but a large amount (~500 ppm) of petroleum (boiling range n-C13 to n-C30)(windowpane flounder, station 115 from the area of *Ocean 250* gasoline spill.)

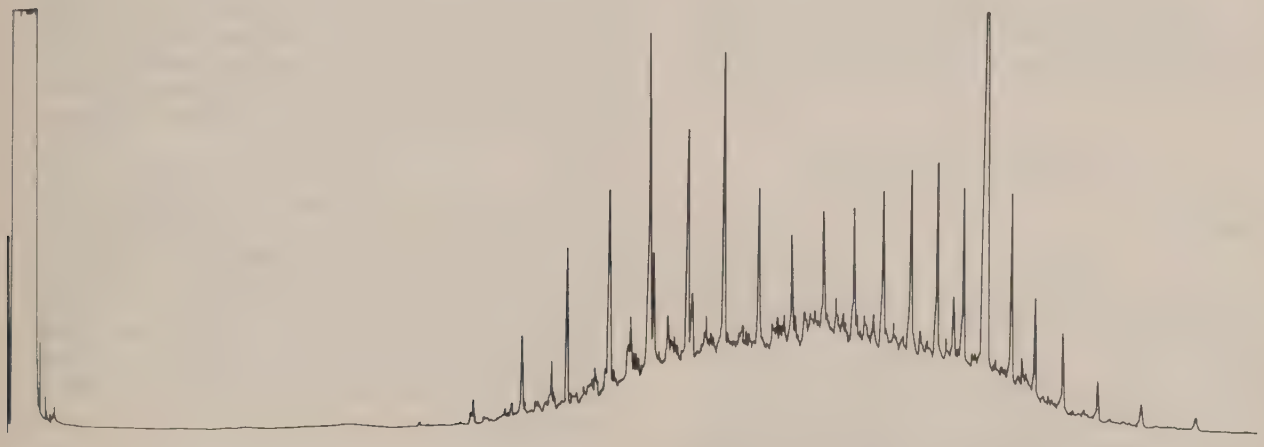


Figure 2-12.—Gas chromatographic trace of yellowtail flounder flesh, station 115 of the *Ocean 250* gasoline spill ~ 400 ppm of petroleum hydrocarbons—no gasoline.

tissues. This would require some more rigorous analytical chemistry (i.e., some GC-MS work). However, the methods and procedures used in this study allow the following conclusions to be drawn:

1. Gasoline consists of alkyl benzenes as a major aromatic hydrocarbon constituent.

2. Components believed to be alkyl benzenes are observed in almost all samples as well as in the control but at low levels (1-2 ppm).

3. Five samples have concentrations of these constituents at levels at least twice the control value although statistical uncertainties in control levels as well as actual control levels of these compounds in species other than winter flounder, *Pseudopleuronectes americanus,* pose interpretative problems.

4. Several samples contain HMW petroleum hydrocarbons at concentrations which are two orders of magnitude higher than the highest suspected gasoline levels.

Acknowledgments.—The laboratory analyses were supported by NOAA Contract No. 02-78-D01-30.

2.5 Summary

On 16 March 1978, the *Ocean 250* spilled 2.6 million liters of gasoline into Block Island Sound. A slick which formed had dissipated within 10 h after gasoline stopped leaking from the damaged barge. On cruises during the following 4 d, samples of water and marine organisms including invertebrates, plankton, and fish were collected for gas chromatographic and GC-MS analyses. Gasoline compounds were found in the water column at concentrations up to 12 μg total gasoline compounds/liter. Low levels of hydrocarbons in the gasoline range were found in some shellfish from the contaminated area. The similarities of gas chromatograms and mass spectra from extracts of shellfish with those from the gasoline standards indicated that compounds from the gasoline spilled by the barge may have been incorporated by some of these benthic organisms.

Gasoline components were found in one plankton sample collected between 1.6 and 0.8 km west of the grounding site and adjacent to stations which contained 10-12 ppb of gasoline in water samples. The gasoline components in this one plankton sample did not match quantitatively the spilled gasoline presumably due to evaporative losses, selective solubilization, and/or selective uptake. Qualitatively, however, the plankton sample contained 18

hydrocarbons in common with a water-accommodated gasoline standard.

Twenty-three samples of flesh from 10 fish species collected in the area of the *Ocean 250* spill were analyzed for gasoline hydrocarbons. Five samples had levels ranging from 1.6 to 2.4 ppm or twice that found in the control sample (0.7 ppm). However, all samples contained some HMW petroleum hydrocarbons; although not quantified, several samples had levels of HMW two orders of magnitude higher than the gasoline concentrations observed.

Despite the volatile nature of gasoline, the water movement due to tides and winds, the consequent redistribution of zooplankton, and the probable movement of fish in and out of the area immediately adjacent to the spill, results of analyses on water, plankton, fish, and the immobile benthic invertebrates show the presence of detectable levels of gasoline components. This indicates a distribution of the gasoline at least for a short time throughout the water column. The low levels found in the shellfish and plankton could reflect a short period of exposure and the possibility of rapid depuration.

The presence of HMW hydrocarbons in many of the fish samples is indicative of a chronic release of petrogenic hydrocarbons into the coastal and marine waters. The impact of this release on fish populations and human health is not known at this time.

3.0 BIOLOGICAL ANALYSES

3.1 Introduction

The impact of oil spills on nearshore marine habitats and renewable resources has been increasingly well documented over the past decade. Much of this information was summarized in the Oil/Environment-1977 Symposium, and since then the impacts of the *Amoco Cadiz* and Campeche spills have been reported. However, these spills and subsequent studies have been of crude or light oil products; there had been no significant gasoline spill in the northeastern United States from which to draw inferences or comparisons of impact on marine communities at the time of the *Ocean 250* spill. However, in the following studies an attempt was made to characterize the species composition and abundance of benthic and zooplankton communities in the area of the spill and to determine if there was any detectable adverse impact by the gasoline on these communities. Additionally fish eggs collected in neuston and plankton nets were examined for cytogenetic damage, since similar analyses of fish eggs collected from areas impacted by various pollutants, including oil, showed increased incidences of abnormal mitotic divisions, development arrest, abnormal patterns of cell differentiation, and other early indicators of embryo death (Longwell 1977; Longwell and Hughes 1989, In press).

3.2 Analysis of Benthic Macrofauna from the Area of *Ocean 250* Gasoline Spill

This section was prepared by Sheldon D. Pratt.

Methods.—Triplicate Shipek grab samples were taken at six stations on a 0.8 km grid previously established by the NMFS (Fig. 3-1). Relatively shallow stations were chosen where dilution of gasoline components through vertical mixing would be minimized.

[12]Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881.

The Shipek grab samples an area 0.04 m^2 and a maximum depth of around 10 cm. Penetration was poor in fine compact sands and samples with a depth of as little as 2 cm were retained for analysis.

Samples were preserved in rose-bengal formaldehyde and sieved to 0.5 mm. For six samples the sediment passing the sieve was saved and combined with residue from faunal sorting for grain size analyses. Dried samples were sieved through a series of 10 screens (2.0-< 0.074 mm) on a ro-tap machine and size fractions weighed.

Fauna were identified to species in most cases. Indicators of recent death or of morbidity were looked for as the fauna was counted.

Results and discussion.

Physical environment.—Station locations and water depths are shown in Figure 3-1 and Table 3-1. The visual appearance of the sediments and grain size distributions are given in Table 3-1 and Figure 3-2.

All of the sediments indicated the dynamic nature of the seafloor in the spill area. Sediment at station 7, in a passage between rock reefs, consisted of rounded, rust-stained gravel and coarse sand washed free of fine sediments by both wave and tidal currents. Sediments at station 17 were a very well sorted fine sand stained a dark rusty brown. This is probably the end product of vigorous and continuous sorting of lower beach material in an oxidizing environment.

The remaining samples were fine sand with gravel and silt. High concentrations of blue mussel shell particles gave these sediments a light gray color. Disaggregating fibrous shell material clouded the water during sieving. The sediments from which blue mussels were recovered consisted of medium sand and a fine sand and silt fraction. Samples 5-1, 5-2, and 7-2 were too silty for sieve analysis. It can be assumed that the fine sediment fraction is a result of the trapping action and biodeposition of the mussel colonies. These fine sediments are probably at least partially swept away by winter storms.

Mussels.—Clusters of the blue mussel were collected in samples 5-1, 5-2, 5-3, and 7-2. The mussels were attached to each

Table 3-1.—Shipek grab sample descriptions (*Ocean 250* spill). One sample from each station was analyzed for grain size. Sample 8-2 was not preserved properly and was discarded. Stations depicted in Figure 3-1.

Station sample	Depth (m)	Sample volume (liter)	Grain size mode (mm)	Visual description
2-1	34	0.75	0.177	gray shelly silty fine sand
2-2		1.0	—	gray shelly silty fine sand
2-3		0.75	—	gray shelly silty fine sand
5-1	9	3.0	—	silty sand with live mussels
5-2		2.0	—	silty sand with live mussels
5-3		0.75	0.5	sand with live mussels
6-1	8	0.8	—	gray shelly fine sand
6-2		0.8	—	gray shelly fine sand
6-3		0.75	0.177	gray shelly fine sand
7-1	9	1.0	—	rounded gravel
7-2		0.75	—	silt, sand, gravel with mussels
7-3		2.0	1.0	coarse sand, gravel
8-1	23	1.3	—	sand, gravel
8-3		1.8	0.25	sand, gravel
17-1	12	1.3	—	brown fine sand
17-2		0.9	—	brown fine sand
17-3		0.75	0.177	brown fine sand

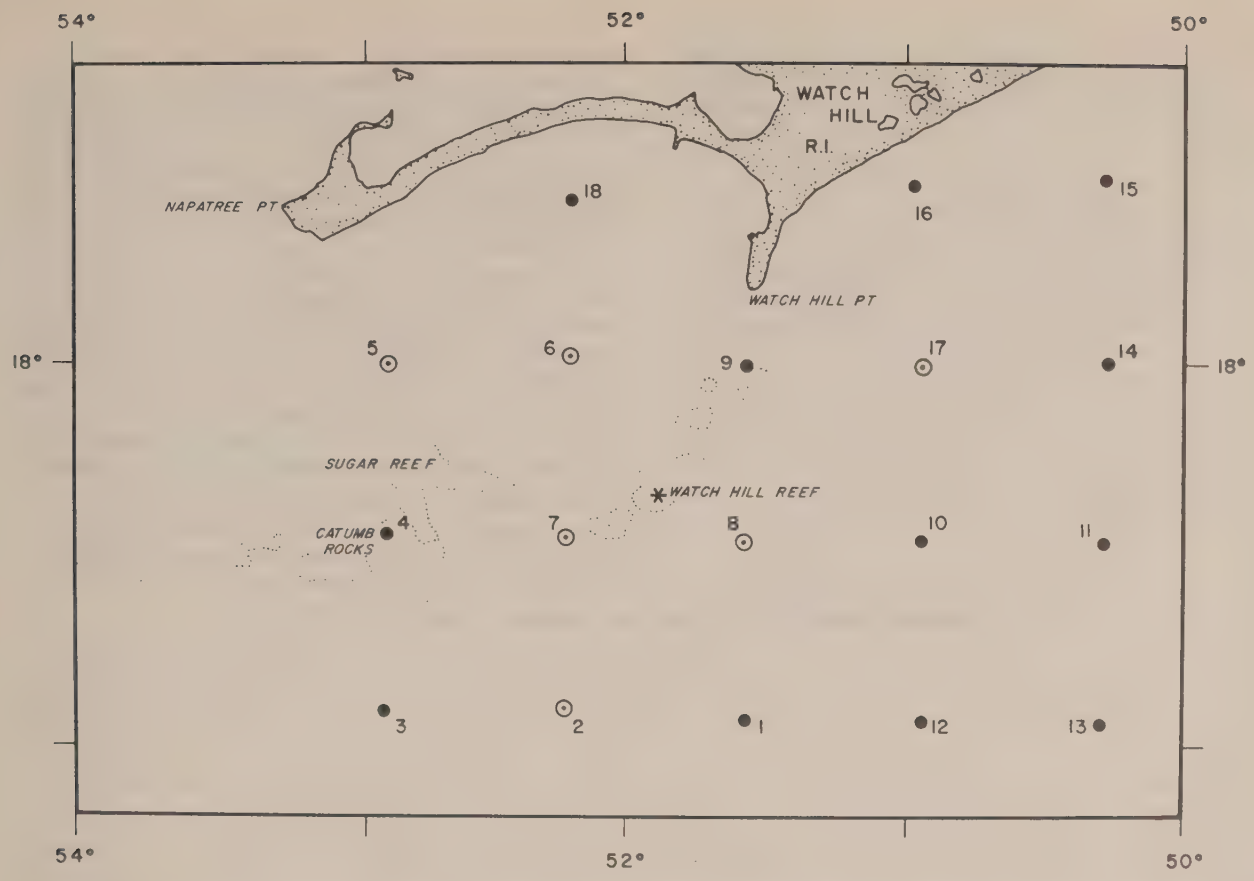
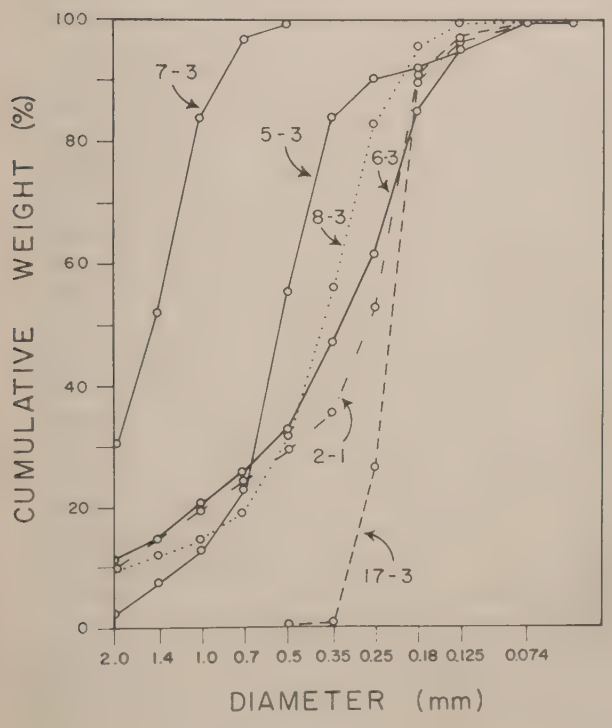


Figure 3-1.—Watch Hill, R.I., survey area. Location of grounding of *Ocean 250* marked by asterisk. Benthic grab sampling stations marked by open circles.



other and not to substrate which was silty sand. All individuals were less than a year old as indicated by their lengths (<4 cm), single size mode, absence of annual growth rings, and absence of fouling organisms. Winter storms may move or bury portions of this soft-bottom population. It is assumed that the extensive rocky substrate in the area supports mussels which reach maturity.

Data on the sizes of live mussels and of shells which are dead but still articulated (clappers) are given in Figure 3-3. Clappers made up a small, relatively consistent, proportion of each collection and did not include the largest animals. Several had been drilled by predatory gastropods. No clappers had tissue in their shells. It is assumed that if a mussel had been killed or weakened by exposure to gasoline within 2 d, tissue would have been present at the time of sampling.

The concentrations of hydrocarbons of 12, 10, and 3 ppb in the water near Watch Hill Reef on 17 March (J. Lake et al., see section 2.2) are well below the 5-50 ppm levels of soluble aromatic hydrocarbons which are actually toxic to tested bivalves (Hyland and Schneider 1976). Gilfillan (1975) commented on the relatively high resistance of blue mussels to acute hydrocarbon exposure. Mussels have been found to accumulate hydrocarbons in their tissues when exposed to high concentrations of oil [1,000-5,000 ppm dry weight in an oil spill area (Straughan 1977); 600-1,200 ppm dry weight near a refinery effluent (Burns and Smith 1977)].

Figure 3-2.—Grain size distributions of representative benthic grab samples from the survey area of the *Ocean 250* spill.

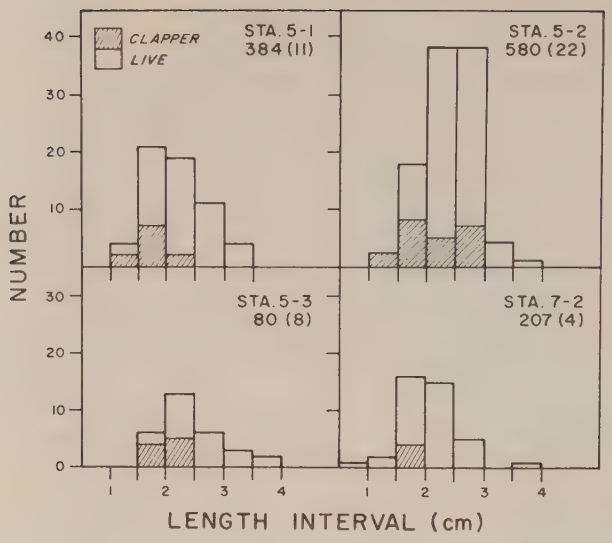


Figure 3-3.—Size class distributions of blue mussels, *Mytilus edulis*, from benthic grab samples. "Clappers" are the shells of dead animals which are still attached. Total number of live and clapper mussels (in parentheses) are shown under sample number. Station locations are indicated in Figure 3-1.

Mussels may show sublethal effects from very low concentrations of hydrocarbons. Gilfillan (1975) found that as little as 1 ppm of the water soluble fraction of crude oil increased respiration while decreasing feeding and assimilation of mussels. Gonzalez et al. (1979) found a decrease in the filter-feeding rate of mussels at concentrations of water-accommodated fraction of No. 2 fuel oil as low as 10 ppb.

It seems likely that exposure to gasoline in the areas sampled (0.8 and 1.6 km from the grounding and at a depth of 10 m) was below acutely toxic levels. Reduced filtering rate or shell closure could also have reduced effective exposure and toxic effects.

Because of the ability of mussels to accumulate hydrocarbons, more extensive collections and analysis may have indicated the horizontal and vertical distribution of gasoline in the spill area. In retrospect it seems that an opportunity was lost to observe the ef-

Table 3-2.—Benthic fauna recovered from Shipek grab samples in the vicinity of Watch Hill Reef (*Ocean 250* spill).

Station-grab	2-1	2-2	2-2	5-1	5-2	5-3	6-1	6-2	6-3	7-1	7-2	7-3	8-1	8-3	17-1	17-2	17-3
Mollusca:																	
Lunatia heros					1									1			
Lunatia triseriata							1										
Mitrella lunata									1								
Nucula annulata	1	2												1			
Mytilius edulis				384	580	80					270						
Musculus sp.			1														
Crenella descussata		1												4			
Astarte undata	1													3			
Astarte castanea												1	1	5			
Crassinella mactracea			1						1				1	1			
Cyclocardia borealis	1	2											1	4			
Arctica islandica													1				
Cerastoderma pinnulatum		1	1														
Spisula solidissima									3				1	1		1	2
Tellina agilis	6	10	10											9		2	1
Periploma sp.													1				
Crustacea:																	
Harpacticoid copepods										1		1		1			
Ostracoda spp.																	2
Chiridotea tuftsi							1								1	4	
Erichsoniella filiformis											1						
Tanaidacea sp.	5		2			2		2	4		1	1	4	4			
Elasmopus levis				1	2	4	1		1		1						
Maera danae					2												
Sympleustes glaber					3	4					6						
Parametopella cypris					1												
Ampithoe sp.										1							
Acanthohaustorius intermedius															1	3	3
Protohaustorius wigleyi															2		3
Bathyporeia parkeri															3		
Phoxocephalus holbolli			3		2	3				1	1						
Paraphoxus epistomus	2	5	1		1	2		1	1	1	2			1	3	1	3
Lysianopsis alba			2		1						1						
Microdeutopus sp.								1		1							
Unciola irrorata	1	1							2								
Erichthonius sp.					1						1						
Corophium sp.						1											
Byblis serrata	43	1											1	4			
Ampelisca vadorum	1		1		2	1								1			
Proboloides holmesi										3							
Pagurus longicarpus													1				
Cancer irroratus				2	2					1				3			
Polychaeta:																	
Lepidonotus squamatus				2	4						1						
Harmothoe extenuata			2	3	9	10				F	27						

fects of very high levels of gasoline on mussels by not using scuba divers to collect them in the immediate area of the grounded barge.

Benthic infauna.

Data.—Counts of benthic fauna recovered from grab samples are given in Table 3-2. Sample 8-2 was improperly preserved and was not sieved. The varying sample volumes mentioned in Table 3-2 should be noted.

Although the species list is long, the populations of this area are of low density and of low biomass with the exception of mussel beds. Many records consist of a single individual and many of the individuals are juveniles or small species.

Condition of infauna.—The condition was assessed by visual means from preserved samples. Little information exists on the characteristics to look for in such an assessment. I have observed animals killed by anoxia and cold temperatures in the field and by high temperatures in the laboratory. When amphipods die the internal tissue breaks down rapidly leaving a transparent "ghost," dead amphipod eggs become cloudy, dead polychaetes are flaccid, and dead bivalves gape. I assume that full guts in amphipods and polychaetes show some degree of normal activity.

The time required for tissue breakdown after death is not known, but it is assumed to be more than 2 d at winter temperatures. I have observed diminished scavenging rates in Rhode Island estuaries at low temperatures. Activity is probably somewhat reduced in open waters as well. A more serious problem in interpreting these results is that many infaunal amphipods, polychaetes, and bivalves leave the sediment when under stress and dead animals could have been washed away from the site of a kill.

No dead animals were observed in these samples. All articulated bivalves were free of tissue remains and most were somewhat weathered. The only visible abnormalities seen were dark

Table 3.2-Continued.

Station-grab	2-1	2-2	2-2	5-1	5-2	5-3	6-1	6-2	6-3	7-1	7-2	7-3	8-1	8-3	17-1	17-2	17-3
Euphrosinid sp.		1															
Eumida sanguinea	1								1								
Phyllodocid sp.										1							
Goniadella gracilis	3			F			F						2		1		
Nephtys picta	1	4	1												2		
Aglaophamus circinatus					F	1						3		4	2	2	3
Nereis diversicolor				2													
Nereis pelagica	1					1					2						
Syliis cornuta										12	1	2	2				
Exogone verugera	6	2	9		3	2	7		3		1		3				
Syllid spp.		1	1			2			F		32				3	1	5
Microphthalmus sczelkowii												5					
Capitella capitata	1		3	29	95	115		1			57						
Mediomastus ambyseta						1											
Clymnella torquata	2	1	2											1			
Travisia carnea															3		
Spio filicornis	1						16										
Spio setosa															3		2
Scolelepis squamata									1				2				
Polydora socialis	4			1	1	4			1		1						
Spiophanes bombyx		1															1
Aricidea jeffreysii	1		2	23	12	1	1	1			2		2	2	2		
Paraonis sp.						3							1				2
Sabellaria vulgaris	3		1										1	1			
Marphysa belli				1							4	2					
Drilonereis sp.						1											
Magelona rosea															2		
Scolopus robustus		F	8											1			
Tharyx acutus	1	1	8	9	19	5					7		9	12			1
Tharyx spp.	1		2	6	2						3		12	4			
Polycirrus medusa								2	1		59	2		1			
Pherusa affinis											1						
Potamilla reniformis	2																
Pisione remota												2					
Echinodermata:																	
Asterias sp.		1				1											
Amphiurid sp.					7	1											
Strongylocentrotus droebachiensis				1													
Other groups:																	
Actiniaria (Anemones)	1	1	1														
Platyhelminthes (flatworms)	1											9					
Rhynchocoela (Nemerteans)	3		3					2				12	F				
Nematoda	13	8	13	4	10	51	22	5	51	1	48	25	19	28		4	
Phascolion strombi (Sipuncula)			1														
Individuals per sample	107	45	79	469	761	295	50	15	72	24	531	65	66	97	28	18	28
Species per sample	27	19	24	15	24	22	8	8	14	11	25	12	20	24	13	8	12
Sample volume (liters)	0.75	1.0	0.75	3.0	2.0	0.75	0.8	0.8	0.75	1.0	0.75	2.0	1.3	1.8	1.3	0.9	0.75

particles adhering to the exterior of an amphipod, *Acanthohaustorius intermedius*, from sample 7-1 and missing but healed appendages in other amphipods.

Benthic assemblages present at the site.—It is useful to group samples according to their faunal composition to aid in recognizing deviations from the norm and in interpreting the effects of the physical environment or fauna.

The most striking faunal assemblage in these samples is that associated with mussel beds (5-1, 5-2, 5-3, 7-2). This includes two species of scale worms, epifaunal amphipods, and a number of deposit-feeding polychaetes (species of *Tharyx, Polycirrus, Aricidea, Capitella*). *Capitella capitata* is often used on an indicator of organic pollution. I find it present in mussel biodeposits in Rhode Island in the winter, presumably responding to the presence of organic sediments rather than pollutants.

Stations outside Watch Hill Reef (2, 8, 17) were characterized by species adapted for unstable sandy bottoms. These species which are widespread in Block Island Sound and the nearshore continental shelf included shallow-burrowing bivalves, free-burrowing amphipods (haustorids and phoxocephalids), a tube-dwelling amphipod (*Byblis* sp.), and free-burrowing nepthid polychaetes and nemertine worms.

The most numerous species in the coarse sand and gravel at station 7 were interstitial sillid polychaetes.

Station 6 had such low numbers of species and individuals that dominants cannot be identified. This poverty of fauna can be contrasted with the abundant fauna at station 5 which is at similar depths 0.8 km to the west, but which was colonized by blue mussels.

Some background data on benthic fauna exists for areas adjacent to the spill site. Biernbaum (1975) examined amphipods at stations off Napatree Point and south of Fishers Island East Point. His samples were large, and he recovered a total of 28 amphipod species from these stations. These appear to be distinct seasonal groups in his collections. Epifaunal and free-burrowing species were found in the winter, and tube builders were found in the summer. This suggests a response to sediment stability. The amphipods found in the spill samples are the same species found in Biernbaum's winter samples.

East Hole, a depression southeast of Fishers Island 37-55 m deep, was studied in detail by Steimle et al. (1976).[13] The presence of a diverse and productive assemblage of tube-dwelling amphipods there illustrates the negative effects of wave exposure on the level bottom benthos in the spill area. It is assumed that depth provides some protection from spills such as this because of the greater volume of water with which water soluble components have to mix and the greater distance that buoyant droplets would have to be vertically advected.

With the exception of blue mussels, the amphipod *Byblis* sp., and a number of small bivalves, most of the dominant infaunal species in the spill area are deposit feeders. Since all feeding types are exposed to water soluble gasoline fractions through respiratory currents, there may be no reason to expect differential effects from a spill of this type.

Very few predators or scavengers were recovered in these samples and so no projections of food web effects can be made.

The northward range of the polychaete *Pisone remota* has been extended from Delaware Bay to Rhode Island by its occurrence at station 17.

Condition of benthic assemblages.—In studies of stressed environments it has been found that there are changes in the density and diversity of benthic fauna and that sensitive species are replaced by more resistant ones. This type of change was not expected at the Watch Hill Reef site because of the limited duration of gasoline exposure. It is possible, however, that a study of infauna data would show that one or more species was absent where expected either due to mortality or escape to the surface.

No examples of such defaunation are detectable from the data of this study. Where species were abundant enough to be sampled adequately, densities are similar in samples from similar habitats. The samples with very low numbers of animals were small in volume and came from areas of sediment instability.

Crustaceans have been identified as especially sensitive to hydrocarbon toxicity, but there are no samples in which this group is obviously reduced.

Adequacy of study.—The samples from shallow areas surrounding the spill site appear adequate to detect any mass mortality of benthic fauna. Either presence of recently dead fauna or absence of fauna from assemblages could have been identified. I consider the time spent separating and identifying infauna worthwhile because it assured that all individuals were examined for visible abnormalities and gave information on the fauna at risk and on the probable physical environment at the site.

Emphasis on immediate mortality rather than long-term biological "imbalances" seems justified in this area. Since the fauna is controlled by substrate instability, it is unlikely that long-term changes due to the relative sensitivity of predators or competitors would be detectable. There may be some species or groups which are sensitive to gasoline fractions and could be rapidly identified and examined. No such animals were identified in this study, however.

It seems likely that the greatest exposure to gasoline must have been on the rocks on which the barge was grounded. However, logistic problems prevented sampling of the epifauna in that area, so an opportunity was lost to learn about the resistance of epifauna to an acute exposure to gasoline in a natural setting. It is possible that in this situation motile epifauna such as amphipods may have left the substrate and indicate gasoline exposure by their absence.

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3.3 Zooplankton Community Structure in the Area of *Ocean 250* Gasoline Spill

This section was prepared by Jerome Prezioso[14] and Carolyn A. Griswold.[14]

Sampling procedure.—Paired 61 cm aluminum bongo frames fitted with 0.333 and 0.505 mm mesh nets were towed in continuous

[13]Steimle, F. W., C. J. Byrne, R. N. Reid, and T. R. Azarovitz. 1976. Hydrology, sediments, benthic macrofauna and demersal finfish of an alternative disposal site (East Hole in Block Island Sound) for the Thames River (Connecticut) dredging project. Final Report. Northeast Fisheries Center Sandy Hook Laboratory, NMFS, NOAA, Highlands, N.J., Informal Report 110, 61 p.

[14]Northeast Fisheries Center Narragansett Laboratory, National Marine Fisheries Service, NOAA, South Ferry Road, Narragansett, RI 02882.

double oblique patterns for 15 min. Nine stations were sampled on 20 March and nine on 10 April (Table 3-3, Fig. 1-2). All samples were preserved in buffered 4% formaldehyde except for the 20 March 0.333 mm mesh samples from stations 13, 14, 15, and 17. These were split: one-half was frozen for hydrocarbon analyses and one-half preserved in formaldehyde. The 0.333 mm net was lost after four stations and the frame was refitted with a 0.505 mm net. Thereafter at stations 3, 5, 6, and 8 one 0.505 mm sample was split and the other was preserved whole.

On 10 April three 0.333 mm samples (stations 1, 11, 14) were split. One-half of each of these was frozen for hydrocarbon analyses and one-half was preserved. The 0.505 mm samples and the remaining 0.333 mm samples were preserved.

Table 3-3.—Summary of plankton samples collected on RV *Strider* cruises 78-01 and 78-02 in the area of the *Ocean 250* grounding. Zero indicates duplicate 0.505 mm mesh samples and no 0.333 mm mesh samples; x indicates 0.333 mm and 0.505 mm mesh samples.

	Plankton	
Station number	RV *Strider* cruise 78-01 20 March 1978 (day 4)	RV *Strider* cruise 78-02 10 April 1978 (day 21)
1		x
3	0	x
5	0	x
6	0	x
8	0	
9		x
10		x
12	x[1]	
13	x	x
14	x	x
15	x	
16		x
17	x	
Total	9	9

[1]0.333 mm mesh net lost; no sample—replaced with 0.505 mm mesh net.

Laboratory procedure.—All fish eggs and larvae were removed from the 20 March and 10 April 0.333 mm and 0.505 mm samples. They were identified, counted, and then sent to Arlene Longwell of the NMFS Laboratory at Milford, Conn., for cytogenetic studies (Hughes and Longwell, see section 3.4).

Each plankton sample was subsampled with a Folsom splitter until the total number of animals in the subsample was reduced to between 500 and 1,000. The zooplankton were identified and counted, and the number of animals per sample was calculated.

Results and discussion.—The species composition is typical of a coastal community with estuarine, coastal, and offshore species. *Oikopleura* sp. was the dominant organism in March following the spill, but was absent in April samples. *Balanus balanoides* nauplii, *Pseudocalanus minutus, Tortanus discaudatus, Temore longicornis,* and *Acartia clausi* characterized both sampling periods. Like *Oikopleura* sp., *Sagitta elegans* numbers varied between the two cruises, but *S. elegans* has been observed to have distinctly patchy distribution. These findings are summarized in Table 3-4 where relative abundance, dominance indices, and elementary statisitics were calculated for the plankton samples from each cruise following the method of Fager and McGowan (1963). For this analysis all plankton samples from each cruise were grouped by mesh size to minimize sampling bias.

The plankton community showed little change during the postspill period. Damage or alteration to the community was not evident. Species composition and abundance at the time just following the spill and 3 wk later remained fairly constant. The only major change in population composition observed was the large number of *Oikopleura* sp. which dominated the plankton samples immediately after the spill, but which was entirely absent in the samples from the follow-up cruise. This is probably attributable to a population bloom and the patchy nature of plankton rather than to an effect of gasoline contamination.

No visual evidence of external hydrocarbon contamination was observed during the identification process.

Acknowledgments.—The authors extend their gratitude to Thomas McKenney (NMFS, Narragansett Laboratory) for his help in the collection of the samples and to Janet Murphy (NMFS, Woods Hole Laboratory) and Joseph Kane (NMFS, Narragansett Laboratory) for analyzing the plankton samples from RV *Strider* cruises 78-01 and 78-02, respectively. We are particularly grateful to Thomas Plichta (NMFS, Narragansett Laboratory) for running the Fager statistical program.

3.4 Cytological-Cytogenetic Analyses of Fourbeard Rockling and Yellowtail Flounder Eggs from Plankton at *Ocean 250* Gasoline Spill

This section was prepared by J. B. Hughes[15] and A. Crosby Longwell.[15]

Methods.—All fish eggs were picked out of the plankton collections and identified as to species. Eggs were those of the fourbeard rockling and yellowtail flounder. Both species have buoyant eggs which float near the water surface (Bigelow and Welsh 1924). Fourbeard rockling eggs were common at more stations throughout the sampling period. As many as 311 fourbeard rockling eggs and only 16 yellowtail flounder eggs were collected. All eggs were used in the following studies.

Eggs were preserved, along with other plankton, in a 1:10 dilution of neutralized formaldehyde. A few of the fourbeard rockling eggs were dehydrated and goldplated for scanning electron microscopy.

To examine the eggs and their chorions in the ordinary light microscope, the chorion was dissected off the egg, the yolk removed and discarded. The embryo and chorion were stained and squashed separately. The stain used was 2% orcein in 45% acetic acid mixed 19:1 with proprionic acid (Longwell and Hughes 1980). Eggs were first sorted as to development stage, and their gross morphology was examined.

Results and discussion.

Condition of chorion, outer egg membrane, of fourbeard rockling eggs.—The outer egg membranes of three fourbeard rockling eggs taken 4 d after the gasoline spill, 20 March, examined with the 100 × objective of the light microscope, showed areas of gross deterioration. Other portions of the same chorions still showed normal structure with pinhole pores. See Figures 3-4 to 3-7. The poor condition of the egg chorion in this small sample of eggs may

[15]Northeast Fisheries Center Milford Laboratory, National Marine Fisheries Service, NOAA, Milford, CT 05460.

Table 3-4.—Relative abundance indices (see Fager and McGowan 1963) for zooplankton from plankton stations, *Ocean 250* spill.

Species	Mean Rank[1]	Dominance[2]	Range[3]	Median[4]	Mean[5]	Dispersion[6]	SD	Frequency[7]	% occurrence[8]
Bongo 0.333 mm zooplankton, 20 March 1978 (RV *Strider* Cruise 78-01)									
Oikopleura sp.	26.00	2/4	26,501-61,331	42,937	43,426.8	6,419.301	16,696.39	4/4	100.0
Balanus sp. nauplii	24.00	0/4	1,892-12,746	5,805	6,562.3	3,148.500	4,545.46	4/4	100.0
Pseudocalanus minutus adult	23.38	0/4	2,524-11,863	5,016	6,105.0	3,044.235	4,311.04	4/4	100.0
Pseudocalanus minutus copepodite	21.00	0/4	442-12,998	2,997	4,858.5	6,360.352	5,558.94	4/4	100.0
Gastropod eggs	20.88	0/4	1,892- 4,795	2,713	3,028.5	633.433	1,385.05	4/4	100.0
Sagitta sp.	20.50	0/4	1,262- 4,038	2,902	2,776.3	657.720	1,351.29	4/4	100.0
Tortanus discaudatus copepodite	20.25	0/4	505- 4,543	2,555	2,539.5	1,290.035	1,809.98	4/4	100.0
Tortanus discaudatus adult	20.13	0/4	442- 3,912	2,933	2,555.3	903.989	1,519.84	4/4	100.0
Acartia sp. adults	18.00	0/4	441- 4,398	1,262	1,840.8	1,712.073	1,775.25	4/4	100.0
Polychaete larvae	17.75	0/4	505- 2,145	1,262	1,293.5	642.924	911.93	4/4	100.0
Invertebrate eggs	17.38	0/4	505- 1,893	851	1,025.3	368.573	614.72	4/4	100.0
Oithona sp.	16.50	0/4	126- 4,291	1,293	1,751.0	1,810.931	1,780.71	4/4	100.0
Temora longicornis adult	13.38	0/4	63- 884	409	441.5	271.028	345.92	4/4	100.0
Centropages hamatus adult	12.25	0/4	252- 378	283	299.3	12.158	60.32	4/4	100.0
Acartia sp. copepodite	10.63	0/4	310- 379	379	267.0	122.629	180.95	3/4	75.0
Medusae	10.13	0/4	252- 379	252	220.8	114.348	158.88	3/4	75.0
Pteropoda	9.38	0/4	126- 883	252	315.3	488.013	392.23	3/4	75.0
Centropages hamatus copepodite	6.63	0/4	63- 252	*	78.8	180.600	119.26	2/4	50.0
Eurytemora herdmani adult	5.75	0/4	252- 252	*	63.0	252.000	126.00	1/4	25.0
Temora longicornis copepodite	5.75	0/4	252- 252	*	63.0	252.000	126.00	1/4	25.0
Cladocera	5.50	0/4	126- 126	*	31.5	126.000	63.00	1/4	25.0
Copepod nauplii	5.50	0/4	126- 126	*	31.5	126.000	63.00	1/4	25.0
Bivalve larvae	5.13	0/4	126- 126	*	31.5	126.000	63.00	1/4	25.0
Eurytemora herdmani copepodite	5.13	0/4	126- 126	*	31.5	126.000	63.00	1/4	25.0
Harpacticus sp.	5.13	0/4	126- 126	*	31.5	126.000	63.00	1/4	25.0
Siphonophora	5.00	0/4	63- 63	*	15.8	63.000	31.50	1/4	25.0
Bongo 0.505 mm zooplankton, 20 March 1978 (RV *Strider* cruise 78-01)									
Oikopleura sp.	33.89	3/9	327-25,176	3,013	6,730.4	11,495.074	8.795.85	9/9	100.0
Sagitta sp.	32.22	0/9	60- 2,902	986	1,266.3	803.357	1,008.62	9/9	100.0
Tortanus discaudatus adult	31.78	0/9	51- 1,451	835	794.2	187.835	386.24	9/9	100.0
Balanus sp. nauplii	30.17	0/9	118- 946	272	402.6	184.049	272.19	9/9	100.0
Tortanus discaudatus copepodite	29.06	0/9	54- 678	213	325.9	142.052	215.16	9/9	100.0
Polychaete larvae	28.78	0/9	15- 568	229	253.1	203.612	227.02	9/9	100.0
Pseudocalanus minutus adult	27.39	0/9	13- 599	189	190.1	185.885	187.99	9/9	100.0
Centropages hamatus adult	25.44	0/9	14- 122	62	61.3	21.461	36.28	9/9	100.0
Temora longicornis adult	24.39	0/9	4- 174	55	57.3	44.080	50.27	9/9	100.0
Gastropod eggs	21.50	0/9	5- 1,293	160	256.0	798.081	452.01	6/9	66.7
Medusae	21.17	0/9	12- 189	63	58.2	62.454	60.30	7/9	77.8
Harpacticus sp.	20.94	0/9	3- 43	16	19.1	9.922	13.77	9/9	100.0
Acartia sp. adult	20.89	0/9	3- 63	31	26.9	15.261	20.26	8/9	88.9
Invertebrate eggs	19.11	0/9	20- 134	63	45.1	49.780	47.39	6/9	66.7
Crangon septemspinosus larvae	18.78	0/9	4- 31	8	12.7	9.928	11.21	8/9	88.9
Gammarus sp.	16.72	0/9	16- 221	39	40.2	124.445	70.75	5/9	55.6
Balanus sp. cypris larvae	14.95	0/9	8- 71	27	14.9	39.013	24.10	4/9	44.4
Unidentified polychaete	13.78	0/9	4- 31	19	8.2	21.186	13.20	4/9	44.4
Oithona sp.	12.44	0/9	8- 31	8	5.2	20.191	10.27	3/9	33.3
Crab zoea	11.50	0/9	3- 16	*	2.1	13.316	5.30	2/9	22.2
Eurytemora herdmani adult	10.83	0/9	8- 8	*	1.8	7.000	3.53	2/9	22.2
Pteropoda	10.72	0/9	1- 4	*	0.6	3.200	1.33	2/9	22.2
Tomopterus sp.	10.72	0/9	1- 8	*	1.0	7.000	2.65	2/9	22.2
Crab megalops	10.33	0/9	63- 63	*	7.0	63.000	21.00	1/9	11.1
Siphonophora	10.06	0/9	39- 39	*	4.3	39.000	13.00	1/9	11.1
Cumacea	9.89	0/9	16- 16	*	1.8	16.000	5.33	1/9	11.1
Copepod nauplii	9.83	0/9	31- 31	*	3.4	31.000	10.33	1/9	11.1
Centropages hamatus copepodite	9.72	0/9	1- 1	*	0.1	1.000	0.33	1/9	11.1
Centropages typicus adult	9.72	0/9	1- 1	*	0.1	1.000	0.33	1/9	11.1
Cladocera	9.72	0/9	1- 1	*	0.1	1.000	0.33	1/9	11.1
Temora longicornis copepodite	9.72	0/9	1- 1	*	0.1	1.000	0.33	1/9	11.1
Bivalve larvae	9.61	0/9	8- 8	*	0.9	8.000	2.67	1/9	11.1
Neomysis americana	9.61	0/9	8- 8	*	0.9	8.000	2.67	1/9	11.1
Pseudodiaptomous coronatus	9.61	0/9	4- 4	*	0.4	4.000	1.33	1/9	11.1

[1]Mean rank: Species were ranked within each sample on the basis of numbers of individuals and ranks for each species were averaged over the samples.
[2]Dominance: The number of samples in which the species made up 50% or more of the individuals.
[3]Range: Smallest and largest nonzero values.
[4]Median: Value for which there are an equal number of nonzero values above and below; * no median calculated because there were <3 values.

Table 3.4—Continued.

Species	Mean Rank[1]	Dominance[2]	Range[3]	Median[4]	Mean[5]	Dispersion[6]	SD	Frequency[7]	% occurrence[8]
Bongo 0.333 mm zooplankton, 10 April 1978 (RV *Strider* cruise 78-02									
Pseudocalanus minutus adult	29.78	3/9	2,182-40,913	12,586	15,020.7	10,132.547	12,336.84	9/9	100.0
Balanidae	28.78	0/9	1,199-14,776	7,109	7,741.1	2,373.956	4,286.85	9/9	100.0
Pseudocalanus minutus copepodite	28.44	0/9	1,643-12,611	5,369	6,320.4	2,411.683	3,904.22	9/9	100.0
Balanidae	24.72	0/9	215- 2,309	495	930.8	624.676	762.52	9/9	100.0
Tortanus discaudatus copepodite	23.50	0/9	137- 4,618	1,238	1,371.3	1,443.494	1,406.95	8/9	88.9
Tortanus discaudatus adult	23.17	0/9	137- 1,099	554	523.4	194.534	319.10	9/9	100.0
Temora longicornis adult	23.00	0/9	206- 1,580	915	827.7	361.774	547.20	8/9	88.9
Temora longicornis copepodite	22.94	0/9	69- 2,049	1,012	859.8	463.537	631.30	8/9	88.9
Acartia tonsa adult	22.44	0/9	55- 2,916	368	699.0	1,510.668	1,027.60	8/9	88.9
Centropages hamatus adult	19.56	0/9	55- 315	109	117.3	67.592	89.05	8/9	88.9
Harpacticoida adult	16.83	0/9	81- 237	151	103.7	83.848	93.23	6/9	66.7
Ammodytes dubius	16.56	0/9	2- 52	23	25.7	10.773	16.63	9/9	100.0
Fish eggs	15.83	0/9	5- 15	9	8.9	0.884	2.80	9/9	100.0
Limanda ferrugine	14.61	0/9	1- 9	3	4.2	2.296	3.11	9/9	100.0
Melanogrammus aeglefinus	14.11	0/9	1- 5	1	2.4	1.341	1.81	9/9	100.0
Centropages hamatus copepodite	11.83	0/9	27- 66	47	20.9	34.078	26.68	4/9	44.4
Medusae	11.67	0/9	69- 462	81	68.0	336.673	151.31	3/9	33.4
Centropages typicus adult	10.67	0/9	17- 79	79	19.4	60.224	34.22	3/9	33.3
Oithona sp. adult	10.56	0/9	34- 44	34	12.4	28.670	18.89	3/9	33.3
Cragonidae	9.83	0/9	54- 55	*	12.1	47.693	24.03	2/9	22.2
Sagitta elegans adult	9.44	0/9	44- 158	*	22.4	124.564	52.88	2/9	22.2
Oithona sp. copepodite	9.39	0/9	44- 44	*	9.8	38.500	19.40	2/9	22.2
Temora stylifera adult	9.39	0/9	44- 66	*	12.2	50.600	24.87	2/9	22.2
Calanus finmarchicus copepodite	8.50	0/9	88- 88	*	9.8	88.000	29.33	1/9	11.1
Acartia longiremus adult	8.39	0/9	185- 185	*	20.6	185.000	61.67	1/9	11.1
Harpacticus spp.	8.39	0/9	131- 131	*	14.6	131.000	43.67	1/9	11.1
Podon sp.	8.33	0/9	100- 100	*	11.1	100.000	33.33	1/9	11.1
Polychaeta	8.22	0/9	92- 92	*	10.2	92.000	30.67	1/9	11.1
Sagitta elegans	8.11	0/9	44- 44	*	4.9	44.000	14.67	1/9	11.1
Gammaridae	8.00	0/9	17- 17	*	1.9	17.000	5.67	1/9	11.1
Bongo 0.505 mm zooplankton, 10 April 1978 (RV *Strider* cruise 78-02)									
Balanidae nauplii	29.94	1/9	13- 3,875	734	1,324.7	1,396.819	1,360.26	9/9	100.0
Tortanus discaudatus adult	29.44	0/9	78- 1,083	624	539.7	166.970	300.18	9/9	100.0
Temora longicornis adult	28.56	0/9	45- 1,027	398	428.2	252.883	329.07	9/9	100.0
Pseudocalanus minutus adult	28.50	0/9	51- 1,250	232	438.2	441.250	439.73	9/9	100.0
Tortanus discaudatus copepodite	27.22	0/9	27- 1,070	140	229.3	452.932	322.29	9/9	100.0
Centropages hamatus adult	25.17	0/9	11- 107	53	59.9	18.933	33.67	9/9	100.0
Ammodytes dubius	22.22	0/9	2- 73	31	30.2	15.500	21.64	9/9	100.0
Balanidae cypris	21.61	0/0	6- 148	33	39.0	49.455	43.92	8/9	88.9
Cragonidae	21.17	0/9	1- 59	11	24.9	20.526	22.60	9/9	100.0
Medusae	20.78	0/9	8- 96	27	31.0	28.831	29.90	8/9	88.9
Fish eggs	20.39	0/9	7- 18	13	13.0	0.846	3.32	9/9	100.0
Sagitta elegans adult	18.11	0/9	8- 79	51	30.2	30.704	30.46	6/9	66.7
Gammaridae	17.44	0/9	3- 39	8	12.6	15.613	14.00	7/9	77.8
Harpacticoida adult	16.78	0/9	3- 133	10	22.2	82.865	42.91	7/9	77.8
Calanus finmarchicus copepodite	14.50	0/0	3- 63	6	11.3	36.529	20.35	6/9	66.7
Melanogrammus aeglefinus	14.22	0/9	1- 5	3	2.7	1.031	1.66	8/9	88.9
Cancer sp.	12.06	0/9	1- 18	10	4.4	12.044	7.32	4/9	44.4
Polychaeta	12.00	0/9	4- 59	21	9.3	42.241	19.86	3/9	33.3
Limanda ferruginea	10.89	0/9	1- 2	1	0.7	0.750	0.71	5/9	55.6
Pseudocalanus minutus copepodite	10.06	0/9	6- 10	*	1.8	7.562	3.67	2/9	22.2
Temora longicornis copepodite	9.67	0/9	3- 18	*	2.3	15.214	5.96	2/9	22.2
Centropages hamatus copepodite	9.56	0/9	4- 5	*	1.0	4.000	2.00	2/9	22.2
Acartia sp. adult	9.28	0/9	1- 4	*	0.6	3.200	1.33	2/9	22.2
Gadus morhua	8.61	0/9	1- 2	*	0.3	1.500	0.71	2/9	22.2
Metridia lucens copepodite	8.56	0/9	32- 32	*	3.6	32.000	10.67	1/9	11.1
Hyperidae	8.44	0/9	20- 20	*	2.2	20.000	6.67	1/9	11.1
Callinectes sapidus	8.39	0/9	11- 11	*	1.2	11.000	3.67	1/9	11.1
Harpacticus spp.	8.17	0/9	5- 5	*	0.6	5.000	1.67	1/9	11.1
Clytemnestra scutellata	8.11	0/9	1- 1	*	0.1	1.000	0.33	1/9	11.1
Cumacea	8.11	0/9	10- 10	*	1.1	10.000	3.33	1/9	11.1
Podon sp.	8.06	0/9	3- 3	*	0.3	3.000	1.00	1/9	11.1

[1]Mean: Arithmetic mean of all station values including zeros.
[6]Dispersion: The ratio of the variance to the mean. The expected value for a random (Poisson) distribution is 1.0.
[7]Frequency: Frequency of occurrence; proportion of samples in which the species was found.
[8]% occurrence: Frequency of occurrence converted to percent.

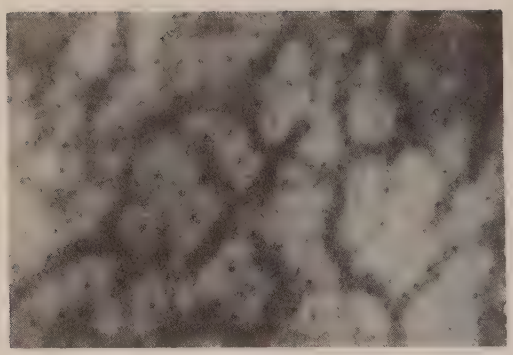

Figure 3-4.—Portion of chorion of fourbeard rockling egg as seen under oil immersion lens (100 ×) of light microscope. Chorion has been removed from egg. Postspill day 4.

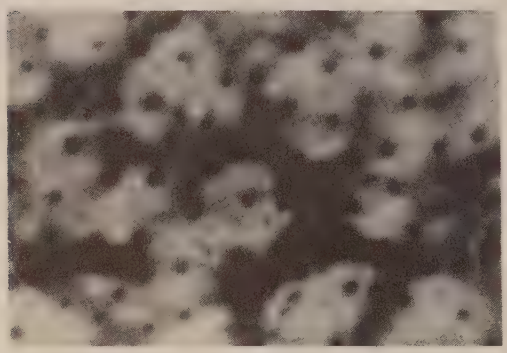

Figure 3-5.—Photographic enlargement of chorion of fourbeard rockling egg seen in Figure 3-4. Regularly spaced black dots are membrane pores (1,000 ×). Postspill day 4.

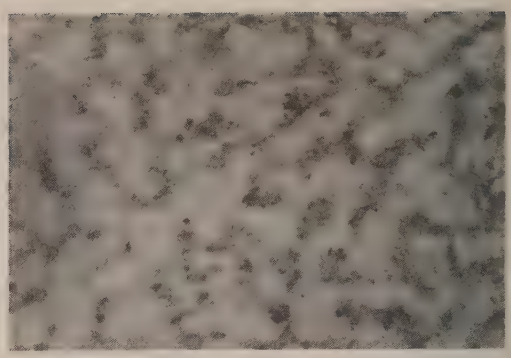

Figure 3-6.—Portion of chorion of fourbeard rockling egg showing deterioration and absence of any pore structure. (100 × immersion objective, light microscope). Postspill day 4.

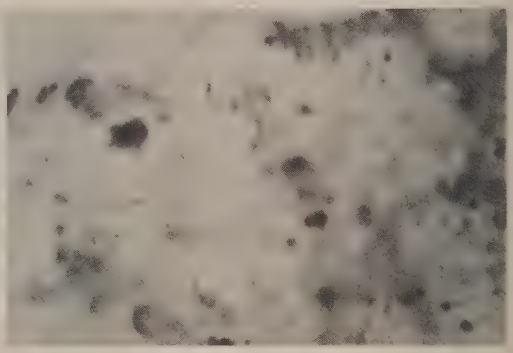

Figure 3-7.—Photographic enlargement of portion of chorion of fourbeard rockling egg showing deterioration and absence of any pore structure (100 × oil immersion objective, light microscope). Postspill day 4.

Figure 3-8.—Intact, undissected fourbeard rockling egg as prepared for examination of chorion in scanning electron microscope and as observed in its entirety under low-power magnification of scanning electron microscope. Pore structure is absent. Lightly shaded portions of egg might represent chorion lesions (1,000 ×). Postspill day 2.

be the result of direct contact of the spawned eggs with gasoline, not merely a postmortem effect. At least some toxic hydrocarbons have a special affinity for membranes, aromatic compounds altering the surface properties of cell membranes (Roubal and Collier 1975). As described below, subsequent cytological examination of the embryos of other eggs from the same samples revealed most of them to be moribund, but only a few embryos were grossly deteriorating at the cell level. Similarly, upon gross examination of the intact eggs, only a very negligible number appeared to be deteriorating. Chorion condition will influence prospects of successful embryo development.

The chorion of another three early-stage fourbeard rockling eggs, from samples taken on 18 March, 2 d after the spill, was examined in the scanning electron microscope. The pattern of pores characteristic of the outer egg membrane of fish (Lønning and Hagström 1975) was completely lacking in these samples. See Figures 3-8 to 3-11.

General morphological and cytological observations on fourbeard rockling embryos from early postspill samples.—Careful examination, prior to dissection, of the fourbeard rockling eggs from samples taken at postspill days 2 and 4, revealed most of them to be partially collapsed. Very few were obviously deteriorating, as noted above. A few had a greenish tinge seldom observed, and of unknown significance, in fish eggs collected in plankton.

In some fourbeard rockling eggs the yolk adhered to the embryo to an extent not previously observed on dissecting thousands of fish eggs of other species. This could have been a hardening effect of the gasoline on the yolk or, less likely, a normal phenomenon for this species.

In a few fourbeard rockling embryos from the second day after the spill the exterior layer of embryo cells had an abnormal appearance. Other cells in the interior of the embryo also occasionally appeared similarly abnormal. Nuclei and mitotic configurations of such embryos often failed to take the stain. A few of these embryos also had abnormally large intercellular spaces with an amorphous material in some spaces and shrunken-appearing cells (Figs. 3-12, 3-13). This has never been observed in prior studies of embryos in varying stages of deterioration. A few fourbeard rockling embryos also had abnormally small, though normal, mitotic configurations.

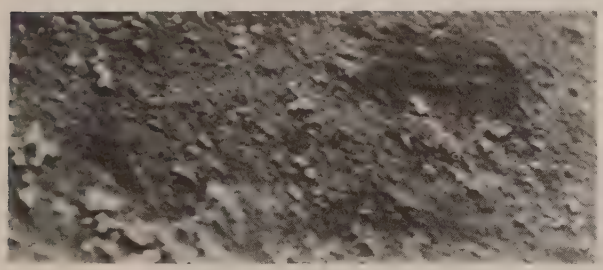

Figure 3-9.—Scanning electron microscope view of a portion of chorion of fourbeard rockling egg. Membrane is deteriorating and no pore structure can be observed. Postspill day 2 (10,000 ×).

Figure 3-10.—Scanning electron microscope view of a portion of chorion of fourbeard rockling egg. Membrane is deteriorating and no pore structure can be observed. Postspill day 2 (25,000 ×).

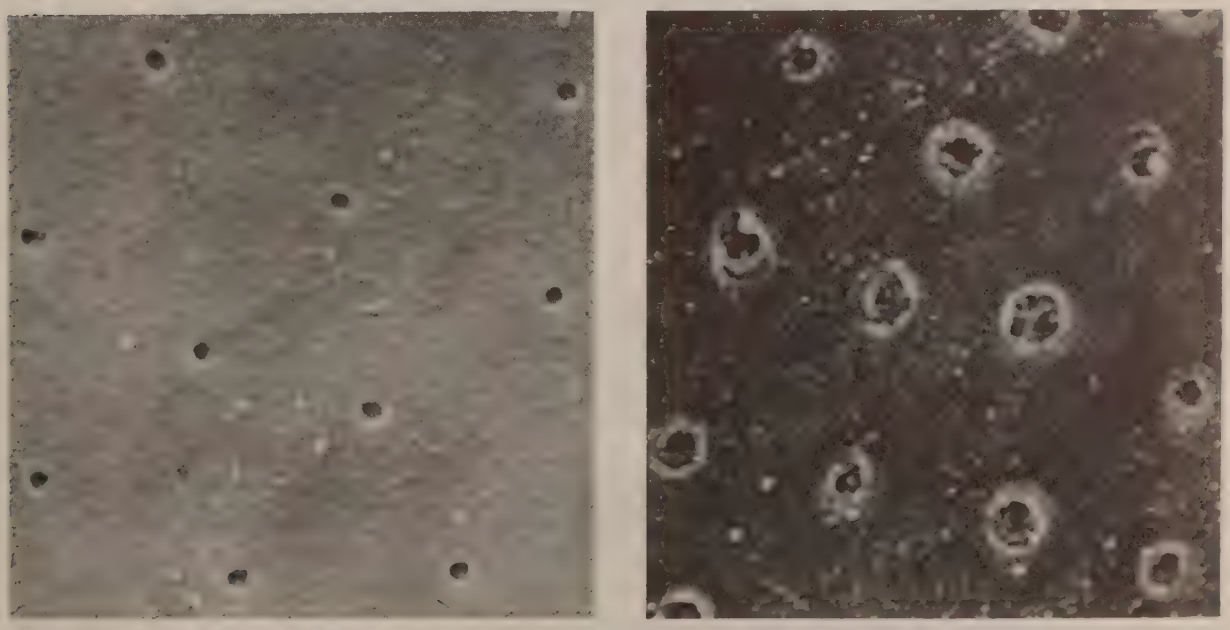

Figure 3-11.—Two scanning electron micrographs (about 10,000 ×), (a) cod, *Gadus callarias*, chorion and (b) pollock, *Pollachius virens*, chorion, are examples of striking pore patterns one expects to find in fish eggs. No such patterns were observable in fourbeard rockling eggs sampled in gasoline spill vicinity 2 d after spill (even though embryo and egg deterioration had not occurred) as demonstrated in Figures 3-9 and 3-10. Compare Figure 3-11 with Figure 3-9 at same magnification.

Figure 3-12.—Normal mitotic divisions in normal Stage II (morula) embryo of silver hake, *Merluccius bilinearis*. Arrows point to two normal mitoses and a normal nondividing cell (100 × oil immersion objective, light microscope).

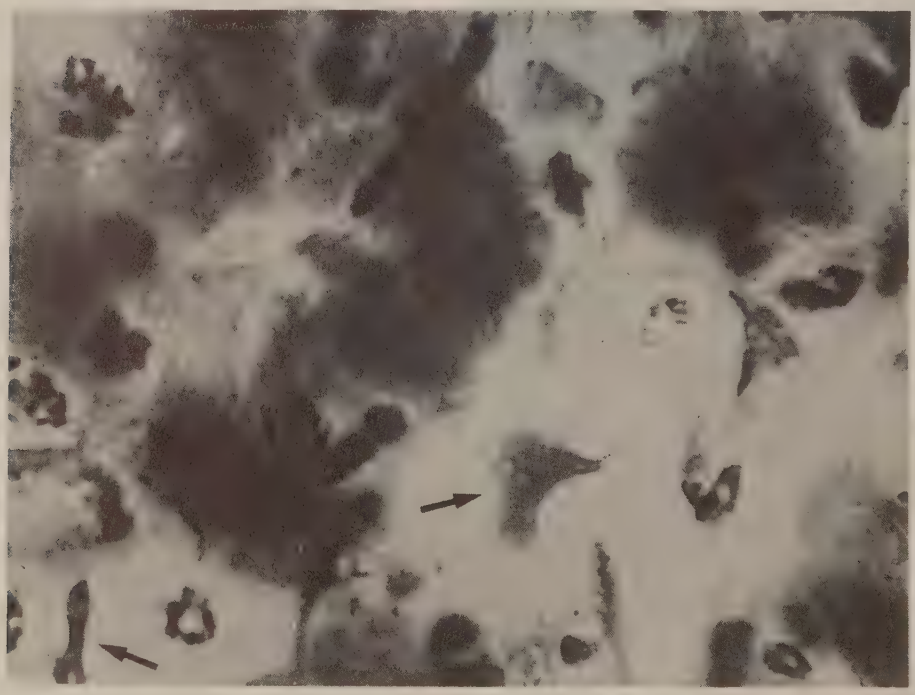

Figure 3-13.—Characteristic pattern of grossly abnormal mitoses (as at upper- and lowermost arrows) and cell deterioration observed in similar stage of the fourbeard rockling eggs from area contaminated by gasoline. Patches of amorphous material remain in wide spaces between shrunken appearing cell groups as at lower middle arrow in Figure 3-13. Note large difference between mitoses as observed in normal Figure 3-12 and this grossly abnormal Figure 3-13. Postspill day 2 (100 × oil immersion objective, light microscope).

Again, such undersized mitotic figures have not been previously observed. It appeared almost as if these embryos had been exposed to a dehydrating agent prior to their fixation, an error that could not have occurred in their processing for microscopic study. Direct exposure of these eggs to the gasoline could have elicited these anomalous phenomena.

Estimates of fish egg moribundity based on cytological-cytogenetic study of the embryos.—Embryo cells and mitoses of all available eggs were examined. Cellular state was determined, and the mitotic index and incidences of abnormal telophases scored over the entire embryo. From these observations estimates of moribundity were made. The fourbeard rockling and yellowtail flounder embryos were categorized as moribund if the embryos showed one or more of the following: cell lysis or nuclear pyknosis; anomalous, disorderly mitoses over the entire embryo (pertinent to stage II only); more than 50% abnormal telophases (pertinent to stage II only); absence of any mitotic telophases whatsoever. Tables 3-5 to 3-8 provide the details of moribundity by developmental stage for each station at which eggs were collected.

Paucity of eggs at several stations did not allow meaningful station-to-station comparisons of moribundity levels. Also, we could not preclude from the station distribution or from the analytical chemical analyses done on plankton, benthos, and fish as reported in this volume, that eggs from any station were not exposed to the gasoline, either by maternal uptake prior to spawning or by direct exposure after spawning. Data are accordingly interpreted and discussed in terms of moribundity for day sampled.

Postspill day 2, 18 March.—(a) Fourbeard rockling. Fourbeard rockling eggs collected 2 d after the spill were, unfortunately, few in number. This, however, may have been the result of egg loss as the most severely affected eggs settle out of the water column, and temporary cessation of much spawning in the affected area in the immediate aftermath of the spill. Total number of eggs in the plankton was 12, and 3 of these were used for scanning electron microscopy, as noted above.

All nine eggs remaining for cytological study of embryo cells and mitoses were moribund (Table 3-5). Eggs were collected at three sample stations only, 112, 115, and 117 (Fig. 3-14). They were all in very early development stages, II and III, i.e., at the morula and blastula stages. The average number of mitotic telophases per embryo, an indicator of developmental rate, was 16.4 even though embryos showed cell lysis and other signs of impending mortality. It is more common to find such moribund embryos with few, or no mitoses at all. It would seem as if the fourbeard rockling eggs were

Table 3-5.—Cytological-cytogenetic estimates of fourbeard rockling egg moribundity 2 d after the *Ocean 250* gasoline spill. See Figure 3-14 (map) for explanation of system for designating same sample station on consecutive cruises.

Sample station	Development stage II-III (morula-blastula)	
	Total no.	No. moribund
112	2	2
115	6	6
117	1	1

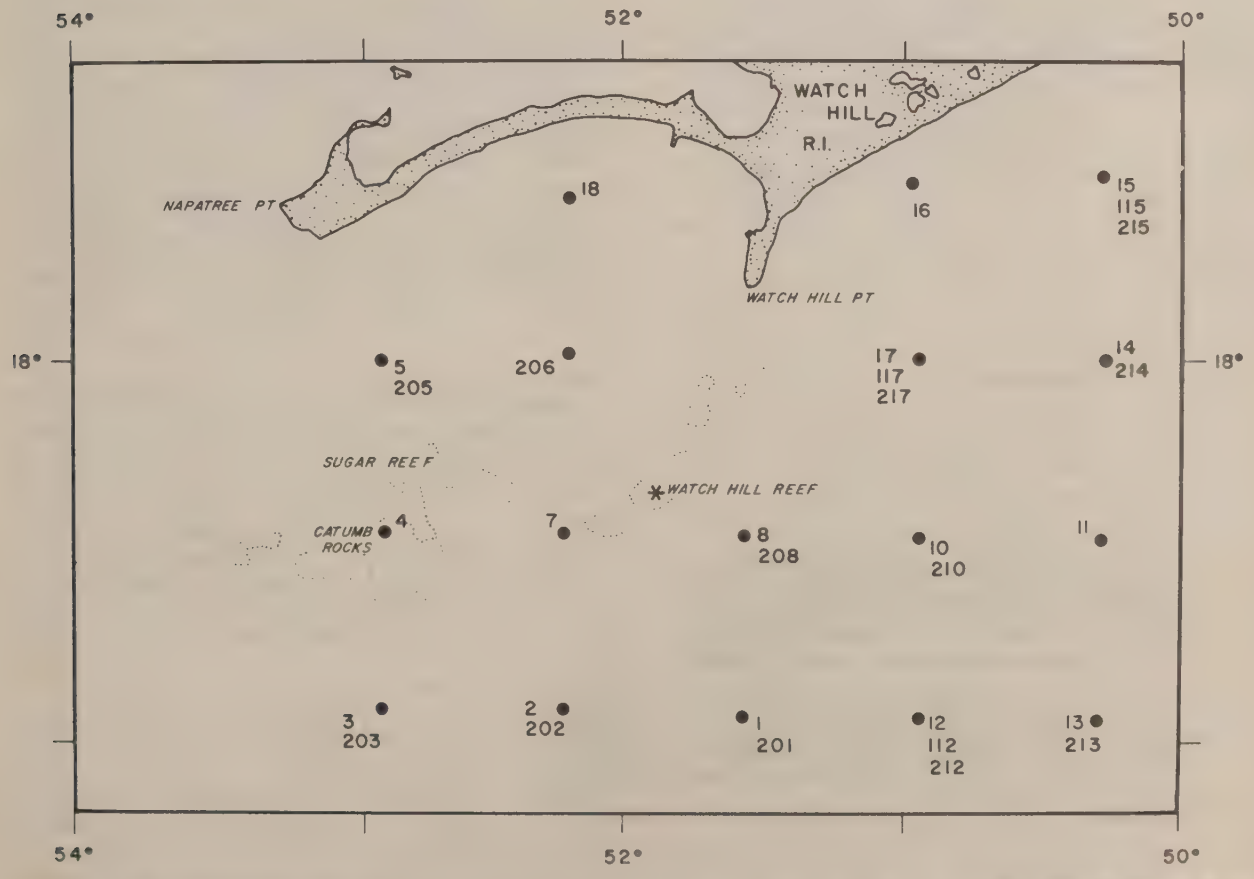

Figure 3-14.—Survey area and station locations for RV *Strider* cruise 78-01. Asterisk marks the location of grounding of barge *Ocean 250* on Watch Hill Reef. The 100 series numbers refer to postspill day 2 samples; the 200 series, to postspill day 4 samples. Numbers 1-18 refer to postspill day 25 samples. Only stations at which fish eggs were sampled are indicated.

developing reasonably well until meeting with some extremely unfavorable conditions.

(b) Yellowtail flounder. No eggs available in samples.

Postspill day 4, 20 March.—(a) Fourbeard rockling. On postspill day 4, more fourbeard rockling eggs and eggs from several additional sample stations (12) were available than on postspill day 2. See Tables 3-5 and 3-6. A portion of all eggs, 31/112 or 27.7%, were at two subsequent developmental stages from those taken 18 March, 2 d after the spill (Table 3-6). Mortality for stages II and III was 59.3% of the eggs (48/81). Mortality for the later stages IV and V (gastrula and early embryo) eggs was less, 7/31 or 22.6%, as might be expected for later, less sensitive developmental stages (Longwell and Hughes 1980, In press). The average number of mitotic telophases was 13.5 for the two earlier stages, and 18.7 for the two later stages. These figures are similar to the 16.4 for the early stages sampled 2 d earlier. See Table 3-6 for data on the 4 d postspill fourbeard rockling eggs.

(b) Yellowtail flounder. Only three yellowtail flounder eggs were sampled at one station (208) on this date and all three were moribund (Table 3-7). They were at stage VI (tail-bud embryo). The average number of mitotic telophases was 10.3, as for the rockling, not markedly low.

Postspill day 25, 10 April.—(a) Fourbeard rockling. Fifteen sample stations are represented in the 10 April collection of fourbeard rockling eggs, three of which had fourbeard rockling eggs on postspill day 2, and most of which had eggs on the 20 March sample date. See Figure 3-14. The number of fourbeard rockling eggs per sample ranged from 1 to 24 (Table 3-8). There were a total of 184 fourbeard rockling eggs available for analyses. Mortality is here again treated by grouping of earlier and later developmental stages since it is expected, on the basis of extensive field data on Atlantic mackerel eggs (Longwell and Hughes 1980), that it would be higher, even naturally, for the earlier stages. For stages II and III moribundity was estimated to be 41.8% (33/79 eggs), somewhat less but not greatly different from the 59.3% of 20 March. For the later stages, IV-VI, the moribundity estimate was 24/105 eggs of 22.9%, hardly any different from the samples taken on postspill day 4.

(b) Yellowtail flounder. Five of the stations yielded yellowtail flounder eggs in the plankton collected 25 d postspill. There was a total of 13 eggs and 10 of these (76.9%) were moribund (Table 3-7). All but one abnormal embryo about gastrulation were at somewhat later development stages than most eggs in this study, stage VI (tail-bud) and stage VII (tail-free). As noted above, the later the development stage, the lower the normally anticipated mortality. This mortality estimate is then high.

Depressed mitotic index of postspill 25 d samples.—The mitotic index taken as the average number of total mitotic telophases per embryo was higher on postspill days 2 and 4 for both yellowtail flounder and fourbeard rockling, early and later development stages, than in was on postspill day 25. As noted above, for yellowtail flounder the average number of telophases was 10.3 in the 20 March sample, but only 1.8 in the 10 April sample (all late stages). For fourbeard rockling it was 16.4 for early stages 2 d postspill. It was 13.5 for early stages and 18.7 for later stages 4 d postspill. At 25 d postspill this number was 6.8 for early stages and 6.1 for later stages. Such a lowering of the rate of cell division in the developing embryos of both fourbeard rockling and yellowtail flounder could be attributable to 1) some interaction of temperature-salinity conditions, but the water should have been warming and development accelerating; 2) some deterioration of water quality not related to the spill; 3) poor quality of eggs spawned subsequent to the spill and its dissipation due to maternal uptake

Table 3-6.—Cytological-cytogenetic estimates of fourbeard rockling egg moribundity 4 d after the *Ocean 250* gasoline spill. See Figure 3-14 (map) for explanation of system for designating same sample station on consecutive cruises.

	Development stage II-III (morula-blastula)		Development stage IV-V (gastrula-early embryo)	
Sample station	Total no.	No. moribund	Total no.	No. moribund
201	7	4	0	0
202	14	7	4	2
203	7	3	1	0
205	5	2	1	0
206	2	1	1	0
208	3	1	0	0
210	9	8	0	0
212	2	0	6	2
213	12	9	2	0
214	5	2	5	1
215	5	4	2	1
217	10	7	9	1

Table 3-7.—Cytological-cytogenetic estimates of yellowtail flounder egg moribundity 4 d and 25 d after the *Ocean 250* gasoline spill—all stages VI (tail-bud) and VII (tail-free) embryos, except one abnormal stage III or IV, in 25 d sample. See Figure 3-14 (map) for explanation of system for designating same sample station on consecutive cruises.

Time of sample	Sample station	Total no. eggs	No. moribund
4 d postspill	208	3	3
25 d postspill	10	6	4
	11	1	1
	13	2	2
	15	2	2
	18	2	1

Table 3-8.—Cytological-cytogenetic estimates of fourbeard rockling egg moribundity 25 d after the *Ocean 250* gasoline spill. See Figure 3-14 (map) for explanation of system for designating same sample station on consecutive cruises.

	Development stage II-III (morula-blastula)		Development stage IV-VI (gastrula-tail-bud-tail-free)	
Sample station	Total no.	No. moribund	Total no.	No. moribund
1	4	3	0	0
2	4	2	3	2
3	9	6	6	4
4	4	3	0	0
6	4	2	11	5
7	3	2	11	6
8	10	3	12	2
10	5	2	7	2
11	5	3	11	0
12	6	2	11	2
13	11	0	13	0
14	0	0	1	0
15	3	1	6	0
16	9	3	13	1
18	2	1	0	0

of gasoline components by fish habitating the area at the time of the spill.

Conclusions.—It would be difficult to interpret the rather anomalous, general cytological findings reported here for the samples taken 2 and 4 d after the spill, except in terms of direct exposure of spawned eggs to the spilled gasoline. It is not surprising that qualitative effects are found on fish eggs that are not simply explainable on the basis of natural variables as temperature and salinity. Chemical analyses of the water column and of plankton and macrobenthos collected during the period, from just after the spill to 4 d after the spill, indicated both the presence of gasoline fractions in the water and its uptake by shellfish (Lake et al., see section 2.2) and plankton (Hoffman and Quinn, see section 2.3) which, of course, would include the fourbeard rockling and yellowtail flounder eggs.

Low number of eggs sampled on postspill day 2 may be attributable to their gross deterioration and settling out of the water column. It is difficult to suppose that the pathologies attributed to direct contact with the gasoline did not increase mortality rates of the fish eggs as sampled 2 and 4 d after the gasoline spill.

The fact that about 40% of the eggs were moribund as long as 25 d after the spill and that their development rate, as measured by mitotic index, was depressed over the 2 and 4 d samples could be interpreted as meaning that the gasoline spill had little to do with the overall estimates of egg mortality. However, it is likely that gasoline components taken up by the fourbeard rockling and yellowtail flounder habitating the area at the time of the spill (Boehm and Barak, see section 2.4) would have affected the quality of the eggs subsequently spawned and sampled in the plankton 25 d after the spill. Unfortunately, no samples of these fish or their spawned eggs were available from the spill area prior to the spill. Also, other contamination in water and biological samples (see papers, this volume) poses problems in the effort to estimate any quantitative impact of the spill.

Yellowtail flounder eggs sampled in the spill area appeared overall to be doing less well than eggs of the fourbeard rockling.

The cytological study of these eggs sampled in the plankton from the area of the gasoline spill and consideration of the significance of the results provide insights that could be useful in planning responses to future spills.

3.5 Summary

Thirty-five grab samples were taken at six stations within 1.6 km of the spill site. The sandy sediments reflected strong wave and tidal currents in the area. Silty sediments were found under dense clumps of blue mussels. Faunal diversity and density was low except in the mussel clumps. However, no recently dead or abnormal mussels or infauna were detected even though gasoline components had been found in other bivalves in the area and no changes in the makeup of faunal assemblages were detectable from the sample counts.

Similarly, analysis of the zooplankton community at 4 d and 3 wk postspill indicated no discernible impact of gasoline, nor was there any visible evidence of damage. Changes that occurred from the 4 d postspill period to the second sampling period were attributed to the patchy nature of plankton and to population blooms rather than to the effect of gasoline contamination.

Bouyant eggs of the fourbeard rockling and yellowtail flounder, sampled 2 and 4 d after the gasoline spill were partially collapsed, had chorion (outer egg membrane) lesions and unusual embryo or egg pathologies. Only 12 eggs, all fourbeard rockling, were collected 2 d after the spill. These were in very early development stages and, on the basis of cytological criteria, were all moribund. Four days after the spill, 84 fourbeard rockling eggs at about the same development stages were close to 60% moribund, using the same criteria. Twenty-five days after the spill moribundity of the 79 fourbeard rockling eggs collected at the same stage was about 40%. In addition, mitotic index, taken as an indicator of development rate and embryo well-being, was depressed over that of earlier samples. Only 16 yellowtail founder eggs were collected in toto over all three sample days, and at somewhat later development stages expected to show lesser mortality than earlier stage fourbeard rockling eggs, however, all but three were moribund.

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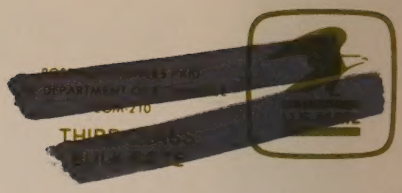

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